(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 2 August 2001 (02.08.2001)

PCT

(10) International Publication Number WO 01/55326 A2

(51)	International Pate	ent Classification ⁷ :	C	12N	60/226,681	22 August 2000 (22.08.2000)	US
					60/227,009	23 August 2000 (23.08.2000)	US
(21)	International App	olication Number:	PCT/US01/0)1347	60/228,924	30 August 2000 (30.08.2000)	US
					60/229,344	1 September 2000 (01.09.2000)	US
(22)	International Fili	ng Date: 17 January	2001 (17.01.2	2001)	60/229,343	1 September 2000 (01.09.2000)	US
					60/229,287	1 September 2000 (01.09.2000)	US
(25)	Filing Language:		Er	nglish	60/229,345	1 September 2000 (01.09.2000)	US
, ,					60/229,513	5 September 2000 (05.09.2000)	US
(26)	Publication Lang	uage:	Er	nglish	60/229,509	5 September 2000 (05.09.2000)	US
()	•			υ	60/230,438	6 September 2000 (06.09.2000)	US
(30)	Priority Data:				60/230,437	6 September 2000 (06.09.2000)	US
(00)	60/179,065	31 January 2000	(31.01.2000)	US	60/231,413	8 September 2000 (08.09.2000)	US
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1	60/227,182	22 August 2000	(22.08.2000)	US	60/239,937	13 October 2000 (13.10.2000)	US
						[Continued on next]	page]

(54) Title: NUCLEIC ACIDS, PROTEINS, AND ANTIBODIES

(57) Abstract: The present invention relates to novel proteins. More specifically, isolated nucleic acid molecules are provided encoding novel polypeptides. Novel polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human polynucleotides and/or polypeptides, and antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to these novel polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting or enhancing the production and function of the polypeptides of the present invention.



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60/259,678	5 January 2001 (05.01.2001)	US

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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

 without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Nucleic Acids, Proteins, and Antibodies

- This application refers to a "Sequence Listing" that is provided only on electronic media in computer readable form pursuant to Administrative Instructions Section 801(a)(i). The Sequence Listing forms a part of this description pursuant to Rule 5.2 and Administrative Instructions Sections 801 to 806, and is hereby incorporated in its entirety.
- [2] The Sequence Listing is provided as an electronic file (PTZ15PCT_seqList.txt, 1,891,228 bytes in size, created on January 13, 2001) on four identical compact discs (CD-R), labeled "COPY 1," "COPY 2," "COPY 3," and "CRF." The Sequence Listing complies with Annex C of the Administrative Instructions, and may be viewed, for example, on an IBM-PC machine running the MS-Windows operating system by using the V viewer software, version 2000 (see World Wide Web URL: http://www.fileviewer.com).

Field of the Invention

[3] The present invention relates to novel proteins. More specifically, isolated nucleic acid molecules are provided encoding novel polypeptides. Novel polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human polynucleotides and/or polypeptides, and antibodies. The invention further relates to diagnostic and therapeutic

methods useful for diagnosing, treating, preventing and/or prognosing disorders related to these novel polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting or enhancing the production and function of the polypeptides of the present invention.

Background of the Invention

- [4] The Human genome is estimated to contain roughly 100,000 genes, each of which plays an important function in sustaining life. Each of these roughly 100,000 genes encodes for a corresponding protein which can be classified based upon its structure and/or function. Some proteins are secreted, while other proteins reside either as membrane associated proteins or intracellularly. Although protein sequences vary substantially, many patterns and overall properties are shared, such as, for example, amino-terminal signal sequences.
- Some proteins, for example secreted proteins, contain an amino-terminal signal [5] sequence which facilitates protein transport. This amino-terminal signal sequence directs, or targets, the protein from its ribosomal assembly site to a particular cellular or extracellular location. Transport may involve any combination of several of the following steps: contact with a chaperone, unfolding, interaction with a receptor and/or a pore complex, addition of energy, and refolding. Moreover, an extracellular protein may be produced as an inactive precursor. Once the precursor has been exported, removal of the signal sequence by a signal peptidase activates the protein. Examples of some protein families that contain signal sequences include cytokines (chemokines) and hormones (growth and differentiation factors). Computer algorithms can be generated to identify amino-terminal signal sequences. Examples of computer programs designed to identify amino-terminal signal sequences include hidden Markov models (HMMs), statistical alternatives to FASTA and Smith Waterman algorithms, which have been used to find shared patterns, specifically consensus sequences (Pearson, W.R., and D.J. Lipman, PNAS, 85:2444-48 (1988); Smith, T.F., and M.S. Waterman, J. Mol. Biol., 147:195-97 (1981)). These algorithms are quite flexible in that they incorporate information from newly identified sequences to build even more successful patterns.
- [6] Other families of proteins exist as membrane associated proteins. Examples of some of these membrane associated protein families include receptors (nuclear, 4

transmembrane, G protein coupled, and tyrosine kinase), protein kinases, phosphatases, neuropeptides and vasomediators, G proteins, ion channels (calcium, chloride, potassium, and sodium), proteases, transporter/pumps (amino acid, sugar, protein, metal and vitamin; calcium, phosphate, potassium, and sodium), matrix molecules (adhesion, cadherin, extracellular matrix molecules, integrin, and selectin), and regulatory proteins. Again, computer programs can aid in the discovery of these molecules. For example, Klein et al. have developed a method ("ALOM", also called as KKD) to detect potential transmembrane segments in polypeptides (Klein, P., et al., Biochim. Piophys. Acta., 815:468 (1985)). It attempts to identify the most probable transmembrane segment from the average hydrophobicity value over a range of amino acid residues. It predicts whether the segment is a transmembrane segment (INTEGRAL) or not (PERIPHERAL), and thus can suggest membrane association of a polypeptide.

- [7] Furthermore, some proteins function intracellularly, and can be identified by their structure and/or function. Computer algorithms can be adapted to aid in the identification of novel members of intracellular protein families. Examples of intracellular proteins include transcription factors, various classes of enzymes, Mitochondrial proteins, and signal transduction molecules.
- [8] Descriptions of some of these proteins (e.g., receptors, hormones, and matrix proteins) and diseases associated with their dysfunction follow.

Summary of the Invention

The present invention relates to novel proteins. More specifically, isolated nucleic acid molecules are provided encoding novel polypeptides. Novel polypeptides and antibodies that bind to these polypeptides are provided. Also provided are vectors, host cells, and recombinant and synthetic methods for producing human polynucleotides and/or polypeptides, and antibodies. The invention further relates to diagnostic and therapeutic methods useful for diagnosing, treating, preventing and/or prognosing disorders related to these novel polypeptides. The invention further relates to screening methods for identifying agonists and antagonists of polynucleotides and polypeptides of the invention. The present invention further relates to methods and/or compositions for inhibiting or enhancing the production and function of the polypeptides of the present invention.

Detailed Description

Tables

Table 1A summarizes some of the polynucleotides encompassed by the invention [10] (including cDNA clones related to the sequences (Clone ID NO:Z), contig sequences (contig identifier (Contig ID:) and contig nucleotide sequence identifier (SEQ ID NO:X)) and further summarizes certain characteristics of these polynucleotides and the polypeptides encoded thereby. The first column provides the gene number in the application for each clone identifier. The second column provides a unique clone identifier, "Clone ID NO:Z", for a cDNA clone related to each contig sequence disclosed in Table 1A. The third column provides a unique contig identifier, "Contig ID:" for each of the contig sequences disclosed in Table 1A. The fourth column provides the sequence identifier, "SEO ID NO:X", for each of the contig sequences disclosed in Table 1A. The fifth column, "ORF (From-To)", provides the location (i.e., nucleotide position numbers) within the polynucleotide sequence of SEQ ID NO:X that delineate the preferred open reading frame (ORF) that encodes the amino acid sequence shown in the sequence listing and referenced in Table 1A as SEQ ID NO:Y (column 6). Column 7 lists residues comprising predicted epitopes contained in the polypeptides encoded by each of the preferred ORFs (SEQ ID NO:Y). Identification of potential immunogenic regions was performed according to the method of Jameson and Wolf (CABIOS, 4; 181-186 (1988)); specifically, the Genetics Computer Group (GCG) implementation of this algorithm, embodied in the program PEPTIDESTRUCTURE (Wisconsin Package v10.0, Genetics Computer Group (GCG), Madison, Wisc.). This method returns a measure of the probability that a given residue is found on the surface of the protein. Regions where the antigenic index score is greater than 0.9 over at least 6 amino acids are indicated in Table 1A as "Predicted Epitopes". In particular embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the predicted epitopes described in Table 1A. It will be appreciated that depending on the analytical criteria used to predict antigenic determinants, the exact address of the determinant may vary slightly. Column 8, "Tissue Distribution" shows the expression profile of tissue, cells, and/or cell line libraries which express the polynucleotides of the invention. The first number in column 8 (preceding the colon), represents the tissue/cell source identifier code corresponding to the key provided in Table 4. Expression of these polynucleotides was not observed in the other tissues and/or cell libraries tested. For those identifier codes in which

the first two letters are not "AR", the second number in column 8 (following the colon), represents the number of times a sequence corresponding to the reference polynucleotide sequence (e.g., SEQ ID NO:X) was identified in the tissue/cell source. Those tissue/cell source identifier codes in which the first two letters are "AR" designate information generated using DNA array technology. Utilizing this technology, cDNAs were amplified by PCR and then transferred, in duplicate, onto the array. Gene expression was assayed through hybridization of first strand cDNA probes to the DNA array, cDNA probes were generated from total RNA extracted from a variety of different tissues and cell lines. Probe synthesis was performed in the presence of ³³P dCTP, using oligo(dT) to prime reverse transcription. After hybridization, high stringency washing conditions were employed to remove nonspecific hybrids from the array. The remaining signal, emanating from each gene target, was measured using a Phosphorimager. Gene expression was reported as Phosphor Stimulating Luminescence (PSL) which reflects the level of phosphor signal generated from the probe hybridized to each of the gene targets represented on the array. A local background signal subtraction was performed before the total signal generated from each array was used to normalize gene expression between the different hybridizations. The value presented after "[array code]:" represents the mean of the duplicate values, following background subtraction and probe normalization. One of skill in the art could routinely use this information to identify normal and/or diseased tissue(s) which show a predominant expression pattern of the corresponding polynucleotide of the invention or to identify polynucleotides which show predominant and/or specific tissue and/or cell expression. Column 9 provides the chromosomal location of polynucleotides corresponding to SEQ ID NO:X. Chromosomal location was determined by finding exact matches to EST and cDNA sequences contained in the NCBI (National Center for Biotechnology Information) UniGene database. Given a presumptive chromosomal location, disease locus association was determined by comparison with the Morbid Map, derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIMTM. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine (Bethesda, MD) 2000. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/). If the putative chromosomal location of the Query overlaps with the chromosomal location of a Morbid Map entry, an OMIM identification number is disclosed in column 10 labeled "OMIM Disease Reference(s)". A key to the OMIM reference identification numbers is provided in Table 5.

[11] Table 1B summarizes additional polynucleotides encompassed by the invention (including cDNA clones related to the sequences (Clone ID NO:Z), contig sequences (contig identifier (Contig ID:) contig nucleotide sequence identifiers (SEQ ID NO:X)), and genomic sequences (SEQ ID NO:B). The first column provides a unique clone identifier, "Clone ID NO:Z", for a cDNA clone related to each contig sequence. The second column provides the sequence identifier, "SEQ ID NO:X", for each contig sequence. The third column provides a unique contig identifier, "Contig ID:" for each contig sequence. The fourth column, provides a BAC identifier "BAC ID NO:A" for the BAC clone referenced in the corresponding row of the table. The fifth column provides the nucleotide sequence identifier, "SEQ ID NO:B" for a fragment of the BAC clone identified in column four of the corresponding row of the table. The sixth column, "Exon From-To", provides the location (i.e., nucleotide position numbers) within the polynucleotide sequence of SEQ ID NO:B which delineate certain polynucleotides of the invention that are also exemplary members of polynucleotide sequences that encode polypeptides of the invention (e.g., polypeptides containing amino acid sequences encoded by the polynucleotide sequences delineated in column six, and fragments and variants thereof).

[12] Table 2 summarizes homology and features of some of the polypeptides of the invention. The first column provides a unique clone identifier, "Clone ID NO:Z", corresponding to a cDNA clone disclosed in Table 1A. The second column provides the unique contig identifier, "Contig ID:" corresponding to contigs in Table 1A and allowing for correlation with the information in Table 1A. The third column provides the sequence identifier, "SEQ ID NO:X", for the contig polynucleotide sequence. The fourth column provides the analysis method by which the homology/identity disclosed in the Table was determined. Comparisons were made between polypeptides encoded by the polynucleotides of the invention and either a non-redundant protein database (herein referred to as "NR"), or a database of protein families (herein referred to as "PFAM") as further described below. The fifth column provides a description of the PFAM/NR hit having a significant match to a polypeptide of the invention. Column six provides the accession number of the PFAM/NR hit disclosed in the fifth column. Column seven, "Score/Percent Identity", provides a quality score or the percent identity, of the hit disclosed in columns five and six. Columns 8 and 9, "NT From" and "NT To" respectively, delineate the polynucleotides in "SEQ ID NO:X" that encode a polypeptide having a significant match to the PFAM/NR database as disclosed in the fifth and sixth columns. In specific embodiments polypeptides of the invention comprise,

or alternatively consist of, an amino acid sequence encoded by a polynucleotide in SEQ ID NO:X as delineated in columns 8 and 9, or fragments or variants thereof.

Table 3 provides polynucleotide sequences that may be disclaimed according to [13] certain embodiments of the invention. The first column provides a unique clone identifier, "Clone ID", for a cDNA clone related to contig sequences disclosed in Table 1A. The second column provides the sequence identifier, "SEQ ID NO:X", for contig sequences disclosed in Table 1A. The third column provides the unique contig identifier, "Contig ID:", for contigs disclosed in Table 1A. The fourth column provides a unique integer 'a' where 'a' is any integer between 1 and the final nucleotide minus 15 of SEQ ID NO:X, and the fifth column provides a unique integer 'b' where 'b' is any integer between 15 and the final nucleotide of SEO ID NO:X, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:X, and where b is greater than or equal to a + 14. For each of the polynucleotides shown as SEQ ID NO:X, the uniquely defined integers can be substituted into the general formula of a-b, and used to describe polynucleotides which may be preferably excluded from the invention. In certain embodiments, preferably excluded from the invention are at least one, two, three, four, five, ten, or more of the polynucleotide sequence(s) having the accession number(s) disclosed in the sixth column of this Table (including for example, published sequence in connection with a particular BAC clone). In further embodiments, preferably excluded from the invention are the specific polynucleotide sequence(s) contained in the clones corresponding to at least one, two, three, four, five, ten, or more of the available material having the accession numbers identified in the sixth column of this Table (including for example, the actual sequence contained in an identified BAC clone).

Table 4 provides a key to the tissue/cell source identifier code disclosed in Table 1A, column 8. Column 1 provides the tissue/cell source identifier code disclosed in Table 1A, Column 8. Columns 2-5 provide a description of the tissue or cell source. Codes corresponding to diseased tissues are indicated in column 6 with the word "disease". The use of the word "disease" in column 6 is non-limiting. The tissue or cell source may be specific (e.g. a neoplasm), or may be disease-associated (e.g., a tissue sample from a normal portion of a diseased organ). Furthermore, tissues and/or cells lacking the "disease" designation may still be derived from sources directly or indirectly involved in a disease state or disorder, and therefore may have a further utility in that disease state or disorder. In numerous cases where the tissue/cell source is a library, column 7 identifies the vector used to generate the library.

Table 5 provides a key to the OMIM reference identification numbers disclosed in Table 1A, column 10. OMIM reference identification numbers (Column 1) were derived from Online Mendelian Inheritance in Man (Online Mendelian Inheritance in Man, OMIM. McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University (Baltimore, MD) and National Center for Biotechnology Information, National Library of Medicine, (Bethesda, MD) 2000. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/). Column 2 provides diseases associated with the cytologic band disclosed in Table 1A, column 9, as determined using the Morbid Map database.

- [16] Table 6 summarizes ATCC Deposits, Deposit dates, and ATCC designation numbers of deposits made with the ATCC in connection with the present application.
- [17] Table 7 shows the cDNA libraries sequenced, and ATCC designation numbers and vector information relating to these cDNA libraries.
- [18] Table 8 provides a physical characterization of clones encompassed by the invention. The first column provides the unique clone identifier, "Clone ID NO:Z", for certain cDNA clones of the invention, as described in Table 1A. The second column provides the size of the cDNA insert contained in the corresponding cDNA clone.

Definitions

- [19] The following definitions are provided to facilitate understanding of certain terms used throughout this specification.
- [20] In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.
- [21] As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence encoding SEQ ID NO:Y or a fragment or variant thereof; a nucleic acid sequence

contained in SEQ ID NO:X (as described in column 3 of Table 1A) or the complement thereof; a cDNA sequence contained in Clone ID NO:Z (as described in column 2 of Table 1A and contained within a library deposited with the ATCC); a nucleotide sequence encoding the polypeptide encoded by a nucleotide sequence in SEQ ID NO:B as defined in column 6 of Table 1B or a fragment or variant thereof; or a nucleotide coding sequence in SEQ ID NO:B as defined in column 6 of Table 1B or the complement thereof. For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having an amino acid sequence encoded by a polynucleotide of the invention as broadly defined (obviously excluding poly-Phenylalanine or poly-Lysine peptide sequences which result from translation of a polyA tail of a sequence corresponding to a cDNA).

[22] In the present invention, "SEQ ID NO:X" was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X is deposited at Human Genome Sciences, Inc. (HGS) in a catalogued and archived library. As shown, for example, in column 2 of Table 1A, each clone is identified by a cDNA Clone ID (identifier generally referred to herein as Clone ID NO:Z). Each Clone ID is unique to an individual clone and the Clone ID is all the information needed to retrieve a given clone from the HGS library. Furthermore, certain clones disclosed in this application have been deposited with the ATCC on October 5, 2000, having the ATCC designation numbers PTA 2574 and PTA 2575; and on January 5, 2001, having the depositor reference numbers TS-1, TS-2, AC-1, and AC-2. In addition to the individual cDNA clone deposits, most of the cDNA libraries from which the clones were derived were deposited at the American Type Culture Collection (hereinafter "ATCC"). Table 7 provides a list of the deposited cDNA libraries. One can use the Clone ID NO:Z to determine the library source by reference to Tables 6 and 7. Table 7 lists the deposited cDNA libraries by name and links each library to an ATCC Deposit. Library names contain four characters, for example, "HTWE." The name of a cDNA clone (Clone ID) isolated from that library begins with the same four characters, for example "HTWEP07". As mentioned below, Table 1A correlates the Clone ID names with SEQ ID NO:X. Thus, starting with an SEQ ID NO:X, one can use Tables 1, 6 and 7 to determine the corresponding Clone ID, which library it came from and which ATCC deposit the library is contained in. Furthermore,

it is possible to retrieve a given cDNA clone from the source library by techniques known in the art and described elsewhere herein. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposits were made pursuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

[24] A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, or the complement thereof (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments described herein), the polynucleotide sequence delineated in columns 8 and 9 of Table 2 or the complement thereof, and/or cDNA sequences contained in Clone ID NO:Z (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments, or the cDNA clone within the pool of cDNA clones deposited with the ATCC, described herein), and/or the polynucleotide sequence delineated in column 6 of Table 1B or the complement thereof. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

[25] Also contemplated are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions

include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH₂PO₄; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

- [26] Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.
- [27] Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).
- The polynucleotide of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.
- [29] The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be

modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth. Enzymol. 182;626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

- [30] "SEQ ID NO:X" refers to a polynucleotide sequence described, for example, in Tables 1Aor 2, while "SEQ ID NO:Y" refers to a polypeptide sequence described in column 6 of Table 1A. SEQ ID NO:X is identified by an integer specified in column 4 of Table 1A. The polypeptide sequence SEQ ID NO:Y is a translated open reading frame (ORF) encoded by polynucleotide SEQ ID NO:X. "Clone ID NO:Z" refers to a cDNA clone described in column 2 of Table 1A.
- [31] "A polypeptide having functional activity" refers to a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein. Such functional activities include, but are not limited to, biological activity,

antigenicity [ability to bind (or compete with a polypeptide for binding) to an antipolypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

- [32] The polypeptides of the invention can be assayed for functional activity (e.g. biological activity) using or routinely modifying assays known in the art, as well as assays described herein. Specifically, one of skill in the art may routinely assay human polypeptides (including fragments and variants) of the invention for activity using assays as described in the examples section below.
- [33] "A polypeptide having biological activity" refers to a polypeptide exhibiting activity similar to, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).
- [34] Table 1A summarizes some of the polynucleotides encompassed by the invention (including contig sequences (SEQ ID NO:X) and clones (Clone ID NO:Z) and further summarizes certain characteristics of these polynucleotides and the polypeptides encoded thereby.

Polynucleotides and Polypeptides of the Invention

TABLE 1A

NO: X (From-To) SEQ	Gene	Clone ID	Contig	SEQ ID	ORF	AA	Predicted Epitopes	Tissue Distribution	Cytologic	OMIM
HFRBN59 1106393 11 1 - 243 335 Pro-1 to Arg-6, AR089: 2, AR061: 1 HFRBN59 1106393 11 1 - 243 335 Pro-1 to Arg-6, AR089: 2, AR061: 1 HEZKI64 906019 12 2 - 778 336 His-1 to Phe-10, AR089: 1, AR061: 1 HAGDV32 1178626 13 247 - 516 337 Pro-1 to Ile-13, AR061: 3, AR089: 3 HAGDV32 1178626 13 247 - 516 337 Pro-1 to Ile-13, AR061: 3, AR089: 3 HAGDV32 238 2 - 250 562 AR061: 13, AR089: 10 HAGDV32 14 2 - 346 338 Ala-47 to Ser-62, L0759: 4, L0777: 3, C10769: 4, L0777: 2, C10		NO: Z		NO: X	(From-To)	SEQ	-	Library code: count	. Band	Disease
HFRBN59 1106393 11 1-243 335 Pro-1 to Arg-6, Glin-38 to Ser-44. 739539 237 77-232 561 Glin-9 to Ser-15. HE2KJ64 906019 12 2778 336 His-1 to Phe-10, Robert Street Pro-25 to Phe-36, Tyr-84 to Trp-90, Pro-25 to Phe-36, Tyr-84 to Trp-90, Pro-25 to Phe-36, Tyr-84 to Trp-90, Pro-55 to Ser-103, Cys-118 to Thr-134. HAGDV32 1178626 13 247-516 337 Pro-1 to Ile-13, Gly-51 to Glin-56, Arg-63 to Thr-68, Ser-75 to Phe-81. 699372 238 2-250 562 HLICC37 856958 14 2-346 338 Ala-47 to Ser-62, Glin-70 to Pro-76.								(see Table IV for		Reference(s):
HFRBN59 1106393 11 1 - 243 335 Pro-1 to Arg-6, Gln-38 to Ser-44. HEZKJ64 906019 12 2 - 778 336 His-1 to Phe-10, Pro-25 to Phe-22, Pro-25 to Phe-36, Pro-25 to Phe						NO: Y		Library Codes)		
HEZKJ64 906019 12 2778 336 His-1 to Phe-10, Asp-17 to Phe-22, Pro-25 to Phe-36, Tyr-84 to Trp-90, Pro-25 to Phe-36, Tyr-84 to Trp-90, Pro-95 to Ser-103, Cys-118 to Thr-134. HAGDV32 1178626 13 247 - 516 337 Pro-1 to Ile-13, Gly-51 to Gln-56, Arg-63 to Thr-68, Ser-75 to Phe-81. 699372 238 2 - 250 562 HI-68, Ser-75 to Phe-81. 699372 238 2 - 250 562 HI-68, Ser-75 to Phe-81. 699372 338 Ala-47 to Ser-62, Glu-70 to Pro-76.	1	HFRBN59	1106393	11	1 - 243	335	Pro-1 to Arg-6,	AR089: 2, AR061: 1		*,
HEZKJ64 906019 12 2778 336 His-1 to Phe-10,		_					Gln-38 to Ser-44.	S0050: 1 and S0260: 1.		
HEZKJ64 906019 12 2778 336 His-1 to Phe-10,			739539	237	77 - 232	561	Gln-9 to Ser-15.			
HAGDV32 1178626 13 247 - 516 337 Pro-17 to Phe-22, Pro-25 to Phe-36, Tyr-84 to Trp-90, Pro-95 to Ser-103, Cys-118 to Thr-134. HAGDV32 1178626 13 247 - 516 337 Pro-1 to Ile-13, Gly-51 to Gln-56, Arg-63 to Thr-68, Ser-75 to Phe-81. 699372 238 2 - 250 562 Arg-63 to Thr-68, Ser-75 to Phe-81. 699372 238 2 - 250 562 Arg-63 to Thr-68, Ser-75 to Phe-81. 699372 238 2 - 250 562 Arg-65 to Phe-81. 699372 238 2 - 250 562 Arg-67 to Ser-62, Glu-70 to Pro-76.		HE2KJ64	ļ	12	2778	336	His-1 to Phe-10,	AR089: 1, AR061: 1		100
HAGDV32 1178626 13 247 - 516 337 Pro-1 to Ile-13, Cys-118 to Thr-134. HAGDV32 247 - 516 337 Pro-1 to Ile-13, Gly-51 to Gln-56, Arg-63 to Thr-68, Ser-75 to Phe-81. 699372 238 2-250 562 HLICC37 856958 14 2-346 338 Ala-47 to Ser-62, Glu-70 to Pro-76.					gup. 444	-	Asp-17 to Phe-22,	L0362: 3, L0794: 2,		
HAGDV32 1178626 13 247 - 516 337 Pro-95 to Ser-103, Cys-118 to Thr-134. Gly-51 to Gln-56, Arg-63 to Thr-68, Ser-75 to Plne-81. G99372 238 2 - 250 562 HLICC37 856958 14 2 - 346 338 Ala-47 to Ser-62, Glu-70 to Pro-76.					-14910-2	4	Pro-25 to Phe-36,	H0624: 1, L0471: 1,		•-
HAGDV32 1178626 13 247 - 516 337 Pro-10 Ser-103, Cys-118 to Thr-134. Gly-51 to Gln-56, Arg-63 to Thr-68, Arg-63 to Thr-68, Ser-75 to Phe-81. 699372 238 2-250 562 HLICC37 856958 14 2-346 338 Ala-47 to Ser-62, Glu-70 to Pro-76.							Tyr-84 to Trp-90,	H0622: 1, H0539: 1,		
HAGDV32 1178626 13 247 - 516 337 Pro-1 to Ile-13, Gly-51 to Gln-56, Arg-63 to Thr-68, Ser-75 to Phe-81. 699372 238 2 - 250 562 HLICC37 856958 14 2 - 346 338 Ala-47 to Ser-62, Glu-70 to Pro-76.					•		Pro-95 to Ser-103,	L0439: 1 and L0581: 1.		
HAGDV32 1178626 13 247 - 516 337 Pro-1 to Ile-13, Gly-51 to Gln-56, Arg-63 to Thr-68, Ser-75 to Phe-81. 699372 238 2 - 250 562 HLICC37 856958 14 2 - 346 338 Ala-47 to Ser-62, Gly-70 to Pro-76.				_			Cys-118 to Thr-134.			
699372 238 2 - 250 562 856958 14 2 - 346 338 Ala-47 to Pro-76.	1	HAGDV32	1178626	13	247 - 516	337	Pro-1 to Ile-13,	AR061: 3, AR089: 3		
699372 238 2 - 250 562 Ser-75 to Phe-81. 856958 14 2 - 346 338 Ala-47 to Ser-62, Glu-70 to Pro-76.							Gly-51 to Gln-56,	L0758: 2, S0010: 1,	,	
699372 238 2 - 250 562 856958 14 2 - 346 338 Ala-47 to Ser-62, Glu-70 to Pro-76.			-				Arg-63 to Thr-68,	L0471: 1 and L0439: 1.		-
699372 238 2 - 250 562 856958 14 2 - 346 338 Ala-47 to Ser-62, Glu-70 to Pro-76.						,	Ser-75 to Phe-81.		•	
856958 14 2 - 346 338 Ala-47 to Ser-62, Glu-70 to Pro-76.			699372	238	2 - 250	295			,	
	i	HLICC37	856958	14	2 - 346	338	Ala-47 to Ser-62,	AR061: 13, AR089: 10		
						•	Glu-70 to Pro-76.	L0769: 4, L0717: 3,		

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74: 3, 29: 2, 56: 2, 50: 1,	42: 1, '8: 1, 18: 1,	14: 1, 13: 1,	48: 1, 70: 1,	73: 1, 59: 1,	53: 1,	59: 1, 48: 1	.c. ±, 50: 1 and		331: 2,	10181: 1	3061: 1	038: 1,	61:1,	21: 1,	30: 1,
20766: 3, L0774: 3, 20775: 3, H0529: 2, 20747: 2, L0756: 2, 20777: 2, H0650: 1,	H0663: 1, S0442: S0358: 1, S0278: 1 H0549: 1, H0318: H0052: 1 1,0738:	H0620: 1, H0014: H0355: 1, H0213:	H0606: 1, S0448: S0142: 1, L0770:	.0646: 1, L0773: .0651: 1, L0659:	1, L0663:	H0547: 1, H0659: 1 H0539: 1 -1 0748: 1	.0750: 1, S0260: 1 and	i	AR089: 1, AR061: H0617: 2, S0031: 2,	S0132: 1 and H0181:	AR089: 1, AR061:	L0766: 4, H0038: 1	H0616: 1, H0561: 1	.0763: 1, H0521:	.0750: 1, L0780:
L0766 L0775 L0747 L0747	H0663 S0358: H0549 H0052	H0620 H0355	H0606: S0142:	L0646: 1, 1 L0651: 1, 1	L0518: 1, 1	H0547	L0750	H0422:	AR085 H061	50132:	AR089	T076	H0616	L0763	L0750
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				,					Ala-61 to Ala-68.	Ala-61 to Ala-68	Pro-101 to Arg-106,	Lys-140 to His-145,	Pro-158 to Val-163.		
		·							Ala-6	Ala-6	Pro-1	Lys-1	Pro-1		
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L0758: 1 and L0595: 1.	AR061: 7, AR089: 5	H0255: 2, L0493: 2	and L0662: 1.			AR089: 4, AR061: 2	L0539: 1 and H0553:		-			AR089: 46, AR061: 9	H0617: 1		-	-			AR061: 3, AR089: 1	H0457: 5,-L0766: 5,	H0581: 2, H0090: 2,	H0521: 2, L0748: 2,	H0171: 1, H0656: 1,	S0212: 1, S0140: 1,	H0486: 1, H0156: 1,	L0471: 1, T0041: 1,
	Arg-14 to Arg-22,	Pro-62 to Ala-79,	Phe-106 to Arg-114,	Glu-120 to Gly-125.		Tyr-47 to His-53,	Lys-87 to Tyr-95,	Ser-110 to Ser-116,	Thr-124 to Ala-129,	Trp-146 to Arg-152.	Tyr-46 to His-52.	Pro-64 to Gly-71,	Lys-101 to Trp-106,	Glu-108 to Gly-116.	His-8 to Gly-18,	Pro-89 to Gly-96,	Lys-126 to Trp-131,	Glu-133 to Gly-141.	Val-30 to Leu-35,	Asn-65 to Leu-71,	Val-144 to Phe-149.	-			-	
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S0344: 1, S0426: 1,	L0655: 1, L0367: 1,	L0792: 1, L0438: 1,	H0690: 1, H0539: 1,	H0436: 1, L0439: 1,	L0779: 1, L0780: 1,	L0755: 1 and H0422: 1.	AR089: 10, AR061: 4	L0759: 2 and H0593:		AR089: 8, AR061: 5	H0677: 54, L0604: 11,	S0366: 7, L0766: 6,	H0445: 6, H0543: 6,	H0556: 5, H0650: 5,	H0255: 5, L0770: 5,	L0655: 5, H0436: 5,	L0777: 5, L0485: 5,	H0657: 4, H0581: 4,	L0769: 4, L0761: 4,	L0747: 4, H0656: 3,	H0599: 3, H0196: 3,	H0373: 3, H0271: 3,	L0520: 3, L0546: 3,	H0423: 3, H0305: 2,	H0333: 2, L0623: 2,	H0457: 2, H0100: 2,
-							Trp-1 to Asp-13.		-																	
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L0763: 2, S0126: 2, H0660: 7, S0330: 7	H0521: 2, S0530: 2, H0521: 2, S0044: 2,	.0751: 2, L0779: 2,	122:	341:	254:	105:	80: 1	587:	186:	H0013: 1, H0002: 1	H0618: 1, H0253: 1	123:)24:	51:]	588:	16: 1	1 13:	525:]	4:1	29: 1	L0762: 1, L0649: 1,	83: 1	28: 1	4:1	701:	570:
, S01	, 200,	, L07	H0542: 2, H0422:	H0583: 1, H0341:	H0484: 1, H0254:	H0306: 1, H0402:	S0354: 1, H0580:	H0586: 1, H0587:	, H0	, H0(, H0	, HO	H0050: 1, H0024:	L0163: 1, H0051:	, H00	S0364: 1, H0616:	, H02	F0041: 1, H0625:	H0561: 1, S0144:	S0422: 1, H0529:	, L06	, L07	, S04	S0053: 1, H0144:	H0698: 1, H0701:	H0699: 1, H0670:
53: 2	21: 2	51: 2	42:2	83: 1	84: 1	06: 1	54: 1	86: 1	59: 1	13: 1	18: 1	18: 1	50: 1	53: 1	16: 1	24: 1,	88: 1	41: 1	61:1	22: 1,	52: 1	40: 1	56: 1	53: 1,	98: 1	99: 1
L07(H05	107	H05	H05	H04	H03	S03,	H05	H05	H00	90H	H03	H00	101	H04	S03	H04	<u>1</u> 007	H05	S04 2	<u> </u>	L05	907	S00	90H	90H
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	is-32,	ly-76,	r-94,	sn-104;	ys-143,	.ys-156,	3lu-175,	Asp-204,	Ser-263,	Hy-291,	sn-320,	Jys-333,	Hu-355,	sn-363,	er-381,	Arg-395,	Asn-441,	he-463,	.ys-490,	3ly-507,	31u-547.	1-7,	0-94	3ly-118,
	Asp-27 to His-32,	Gln-65 to Gly-76,	Lys-80 to Ser-94,	Pro-99 to Asn-104,	Gly-126 to Lys-143,	Pro-150 to Lys-156,	Glu-163 to Glu-175,	Val-193 to Asp-204,	Met-230 to Ser-263,	Ala-278 to Gly-291,	Pro-306 to Asn-320,	Asn-328 to Lys-333,	Glu-348 to Glu-355,	Ile-358 to Asn-363,	Glu-375 to Ser-381	Lys-390 to Arg-395,	Lys-433 to Asn-441,	Ser-456 to Phe-463,	Glu-484 to Lys-490,	Glu-498 to Gly-507,	Glu-535 to Glu-547	Arg-1 to Ala-7,	Thr-75 to Pro-94,	Arg-111 to Gly-118,
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Asp-122 to Gln-130.	His-1 to Glu-14,	Asp-26 to Lys-34,	Ser-47 to Lys-52,	Asn-97 to Gly-107,	Lys-123 to Gln-129,	Glu-215 to Asp-228,	Pro-245 to Glu-250,	Leu-255 to Glu-260,	Glu-275 to Gly-306.	٠				2	-												
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	Asn-58 to Gly-64. Asn-58 to Gly-64.	Glu-1 to Lys-27, Thr-77 to Leu-82, Asp-114 to Lys-119, Ser-130 to Thr-138.	Gly-1 to Glu-12, Glu-22 to Gly-35, Pro-37 to Thr-49, Tyr-72 to Asn-81, Arg-191 to Asp-196, Gly-211 to Thr-218, Ala-256 to Asn-261, Gln-269 to Phe-282, Leu-286 to Arg-293, Phe-393 to Asp-400, Thr-407 to Thr-414.	Gly-1 to Glu-12,
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Glu-22 to Gly-35,	Pro-37 to Thr-49.	Ser-40 to Ser-45,	His-75 to Trp-81,	Ser-113 to Lys-128,	Pro-146 to Thr-154,	Asp-217 to Val-229,	Gly-261 to Gln-270,	Glu-313 to Thr-319,	Pro-346 to Leu-359,	Ala-378 to Ser-385,	Ser-388 to Asn-393,	Val-407 to Asp-418,	Ser-422 to Leu-428,	Thr-431 to Leu-441,	Leu-478 to Ala-489,	Gly-499 to Pro-522,	Glu-527 to Tyr-535,	Glu-540 to Arg-550,	Arg-560 to Arg-593,	Arg-625 to Ile-630,	Gln-642 to Tyr-649,	Lys-669 to Met-675,	Ala-687 to Thr-706,	Thr-734 to Asn-739.	Pro-78 to Lys-86,	Cys-88 to Leu-97,	Asp-100 to Ile-107,
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Pro-176 to Pro-181,	Arg-191 to Met-196,	Pro-200 to Arg-210,	Pro-246 to Ala-259,	Ser-271 to Glu-276,	Asp-298 to Trp-306,	Pro-332 to Ser-340.					•		-		•	•	09					,	-				
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				Asn-7 to Asn-12.	·	Glu-19 to Asn-42,	Ala-135 to Gly-140.	Ser-21 to Leu-26,	Leu-63 to Ser-76,	Ala-141 to Pro-153,	Pro-184 to Leu-194,	Gln-235 to Gly-240,	Pro-279 to Asp-288,	Lys-296 to Pro-302.	•					•				Gly-9 to Gly-18,	Arg-23 to Leu-28,	Leu-65 to Ser-78,	Ala-143 to Pro-155,
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	Pro-186 to Leu-196,	Gln-237 to Gly-242,	Pro-281 to Asp-290,	Lys-298 to Pro-304.	Glu-14 to Ala-21,	Lys-51 to Ser-59,	Ile-70 to Phe-75,	Ala-107 to Arg-113,	Thr-124 to Asn-131,	Tyr-171 to Asn-176,	Gln-187 to Asn-238,	Ser-243 to Ile-248,	Glu-265 to Ser-271,	Pro-281 to Glu-298,	Ser-309 to Met-316,	Pro-321 to Pro-329,	Gln-374 to Arg-381,	Asp-390 to Cys-400.								•	-	
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Ser-86 to Ser-92.	Val-12 to Gln-17, Ala-75 to Arg-82, Lys-112 to Ile-117, Asn-179 to Trp-185, Asn-190 to Lys-209.				
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S0214:1, H0428:	H0111: 1, H0165:	.0455: 1, H0090:	H0634: 1, H0616:	H0551: 1, L0564:	H0641: 1, H0646:	S0144: 1, S0422:	H0695: 1, L0521:	.0767: 1, L0804:	L0656:	0790: 1, L0791:	1, L0438:	S0126: 1, H0435:	H0648: 1, H0539:	H0522: 1, H0631:	S0028: 1, L0747:	.0731: 1, L0759:	.0583: 1, S0011:	H0136: 1, S0192:	S0276: 1, H0543:	[pui	AR089: 3, AR061:	0, L	H0620: 7, L0747: 7,	.0637: 5, H0265: 4,	H0013: 4, H0551: 4,	0,0
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H0085.1		62: 1,]	.66: 1,	H0292: 1,	40: 1,	90: 1,	H0038: 1,	H0433: 1,	S0038: 1,	S0352: 1,	S0142: 1,	L0761: 1,	.0646: 1,	.0764: 1,	_	L0388: 1,			<u>_</u>		H0689: 1,	H0660: 1,	H0696: 1,	S0037: 1,	06: 1,	54: 1,	.0750: 1,
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	Gln-18 to Gly-32, Val-84 to Asn-91.				•			•								1			,						:
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		Leu-68 to Gln-77.	Gly-1 to Arg-7,	Ala-9 to Ser-15,	Ala-25 to Gly-30,	Gln-75 to Cys-84,	His-111 to Tyr-116.	•		•							-			•		-			Gly-10 to Lys-17.	Thr-10 to Ala-21,
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Gln-35 to Trp-45, Gly-54 to Leu-61.	, ,			,		Pro-30 to Gly-35.					•	Ser-6 to Glu-16,	Asp-33 to Lys-38,	Glu-71 to Phe-79,	Gln-120 to Glu-131,	Met-152 to Asp-159,	Ala-169 to Pro-174,	Leu-182 to Lys-201.		Pro-35 to Ser-43,	Glu-61 to Phe-69,	Gln-110 to Glu-120.	Gln-12 to Gln-17,	Arg-64 to Thr-69,	Ser-127 to Ser-132.
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	Thr-27 to Leu-32, Gly-42 to Gly-56, Ser-80 to Arg-90.	Phe-14 to Pro-20, His-23 to Ile-30, Ala-53 to Thr-58.	Thr-108 to Gly-115, Val-174 to Gly-181, Ala-205 to Gly-214, Pro-272 to Asn-282.	Pro-1 to Glu-13, Ser-22 to Lys-28, Gln-39 to Arg-50, Ser-111 to Asp-116.	
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		Pro-5 to Lys-22, Arg-43 to Glu-51,	Arg-63 to Ala-71,	Asp-/3 to Lys-/9.							,								4.4.						w-1	
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His-44 to Gly-49,	Pro-1 to Arg-8.
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	Thr-16 to Thr-22, Leu-29 to Met-37, Pro-55 to Gln-64, Ser-69 to Leu-75, Pro-82 to Ser-95, Lys-126 to Val-142, Ser-159 to Leu-172, Arg-174 to Met-181, Thr-189 to Asn-195, Hag-216 to Trp-229, Leu-266 to Gly-272, Hal-283 to Glu-289, Leu-265 to Glu-289,
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Cl. 210 to I on 215	Our-210 to Lett-213.		-	•	•									×		Pro-3 to Gly-11,	Gly-53 to His-63,	Leu-70 to Lys-89,	Met-99 to Thr-108.	Gly-1 to Lys-7.		Leu-39 to Arg-44.		•			
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	Leu-39 to Arg-44, Lys-178 to Asp-186.		Glu-20 to Ala-30,	Ser-75 to Gln-83,	Gly-148 to Gly-154,	Arg-158 to Ser-167,	Pro-169 to Pro-176,	Leu-213 to Val-222.	Gln-5 to Gly-10.	Arg-12 to Gln-23,	Asp-82 to Pro-88,	Gly-112 to Ala-120,	Arg-122 to Arg-127,	Gly-172 to Gly-186,	Val-212 to Gly-219,	Gly-242 to Gly-247,	Thr-253 to Ser-265.			
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	Ala-29 to Cys-34.	Ser-14 to Val-23,	Lys-76 to Ser-84,	Ser-102 to Leu-109,	Gln-119 to Cys-125,	Glu-177 to Thr-189,	Ala-221 to Phe-231.	•								•			
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															Glu-3 to Phe-8,	Lys-43 to Glu-48,	Gly-62 to Pro-71.	Glu-3 to Phe-8,	Lys-43 to Glu-48,	Gly-62 to Pro-71.	Ala-94 to Cys-100,	Ser-126 to Val-136,	Val-161 to Asn-166.	Ala-94 to Cys-100.	Pro-5 to Lys-12,	Pro-18 to Arg-37,	Asn-56 to Gly-63,	Ser-75 to Arg-83,
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Gly-147 to Gly-156.		. 181				-							Ala-8 to Gly-13,	Gln-58 to Cys-67,	His-94 to Tyr-99,	Ser-107 to Ala-112.	Glu-29 to Leu-37,	Ser-47 to Glu-53,	Glu-87 to Gln-92,	Asn-112 to Ala-119.						•	
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	H0581:	H0123: 1	L014	S0003:	H0039:	H0030:	H0598: 1	L0060: 1	S0015:	S0440:	H0646:]	S0210:	L0378:]	S0374:	H054	H0436: 1	L077	S0260:	L0596: 1	L0485: 1	L0593:	H0653: 1	AR0	H02	L059	H01 ²	H001	L076
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H0539: 2, L0750: 2, L0777: 2, L0758: 2, L0759: 2, S0242: 2, S0424: 2, H0624: 1, S0040: 1, S0420: 1, L0005: 1, S0356: 1, H0009: 1, H0570: 1, S0051: 1, H0038: 1, H0413: 1, T0069: 1, L0649: 1, L0794: 1, L0650: 1, L0794: 1, L0666: 1, H0520: 1, H0666: 1, H0520: 1, H0666: 1, H0514: 1, S0028: 1, L0439: 1,	AR089: 0, AR061: 0 H0090: 2, H0052: 1 and L0439: 1.	AR061: 9, AR089: 7 L0766: 3, H0457: 2, H0551: 2, H0529: 2, L0527: 2, H0144: 2, S0152: 2, H0521: 2, L0759: 2, H0343: 2,
		Gly-1 to Trp-7, Gln-47 to Ser-54, Glu-105 to Asn-110, Thr-115 to Asp-123, Glu-147 to Asp-152, Glu-161 to Asn-167,
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H0542: 2, H0624: 1,	H0306: 1, H0619: 1,	L0586: 1, H0013: 1,	H0635: 1, H0327: 1,	H0615: 1, H0591: 1,	S0002: 1, L0796: 1,	L0805: 1, L0791: 1,	L0745: 1, L0750: 1,	L0780: 1, L0731: 1,	L0599: 1 and H0422: 1.	AR061: 9, AR089: 4	L0731: 8, L0803: 4,	L0665: 3, L0756: 3,	S0358: 2, L0637: 2,	L0662: 2, L0666: 2,	L0777: 2, L0595: 2,	H0170: 1, H0662: 1,	L0005: 1, S0222: 1,	H0409: 1, H0486: 1,	S0388: 1, H0428: 1,	H0561: 1, S0450: 1,	H0538: 1, H0529: 1,	L0800: 1, L0764: 1,	L0794: 1, L0766: 1,	L0774: 1, L0659: 1,	L0783: 1, L0663: 1,	L0664: 1, H0521: 1,	H0436: 1, S3014: 1,
Pro-188 to Lys-195.										Arg-1 to Glu-8,	Ser-249 to Glu-254.												-		-		
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				Leu-88 to Pro-94,	His-164 to Pro-174.					• -2	-						-				٠.						
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	Ser-10 to Glv-15,	Pro-20 to Ser-27,	Glu-34 to Gly-41,	Ala-45 to Trp-50,	Pro-79 to Gly-88.				Ile-30 to Gly-36,	Thr-67 to Thr-72.	Gly-15 to Arg-21,	Pro-30 to Ser-35,	Ser-44 to Asp-51,	Pro-109 to Phe-115,	Glu-131 to Ser-139,	Arg-166 to Ser-179,	Gly-205 to Gly-215,	Ser-234 to Arg-252,	Arg-279 to Glu-288,	Leu-355 to Cys-362,	Glu-371 to His-376,	Thr-393 to Asp-401,	Arg-506 to Asn-512,	Asp-571 to Lys-578,	Pro-580 to Pro-592,
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L0779: 1, H0543: 1 and H0506: 1.			AR089: 15, AR061: 2	S0126: 4, H0135: 3,	H0494: 3, H0547: 3,	S0045: 2, H0550: 2,	H0545: 2, H0242: 2,	H0266: 2, H0551: 2,	H0653: 2, S0040: 1,	S0282: 1, S0358: 1,	S0376: 1, S0046: 1,	H0393: 1, S6022: 1,	H0549: 1, H0156: 1,	H0618: 1, H0253: 1,	H0123: 1, H0050: 1,	H0024: 1, H0014: 1,	H0252: 1, H0124: 1,	H0040: 1, H0623: 1,	S0370: 1, S0210: 1,	L0648: 1, L0518: 1,	S0374: 1, H0435: 1,	S0328: 1, S0152: 1 and		
Lys-601 to Leu-608.	Ser-9 to Asp-16,	Pro-74 to Phe-80, I ys-85 to Glv-91	Ser-26 to Val-32,	Ala-60 to Trp-66.									•										Pro-6 to Arg-14,	Gly-97 to Asp-109,
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Pro-118 to Cys-123, Cys-135 to Ser-140.	Pro-9 to Arg-17, Glv-100 to Asn-112	Pro-121 to Cys-126,	Cys-138 to Ser-143.	Ser-75 to Lys-80,	Arg-167 to Lys-172.				-										~					*	,
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					67,	Arg-282 to Arg-289,	15,	Arg-375 to Gln-381,	99,	9																	
			Ser-15 to Cys-21,	Leu-52 to Ser-58,	Gly-161 to Glu-167,	. 2 -2	Ser-340 to Gln-345,	11-3	Gly-392 to Ala-399,	Pro-401 to Trp-406.					•												
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Gly-5 to Arg-12, Ile-52 to Thr-61, Val-85 to Gly-92, Tyr-114 to Thr-121, Lys-133 to Pro-138, Thr-186 to Arg-192.	Arg-1 to Ser-14, Glu-46 to Glu-51.	Ala-1 to Gly-12.
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	Lys-93 to Gln-98,	Asp-141 to Leu-148,	Asn-166 to Pro-172,	Glu-174 to Gln-179,	Ser-187 to Lys-192,	Gln-221 to Gln-229,	Pro-239 to Asp-246.	•				-		,		-											
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	Val-51 to Arg-56, Ala-127 to Asp-133, Val-147 to Glu-153.	Gly-8 to Cys-13, Gln-38 to Met-48, Arg-76 to Gln-82, Cys-87 to Asp-94.	Cys-22 to Cys-31, Leu-35 to Pro-54, Gln-59 to Glu-73, Arg-131 to Met-140, Asn-149 to Arg-156,
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Ser-191 to Thr-196.	Leu-299 to Leu-313,	Glu-328 to Pro-336,	Val-393 to Asp-399,	Asn-454 to Asn-466.																		•					
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													Pro-1 to Asp-10,	Arg-24 to Leu-42,	Val-65 to Ser-75,	Arg-95 to Asp-104,	Glu-111 to Ile-119,	Asp-151 to Arg-157,	His-212 to Leu-222,	Ala-251 to Gly-256,	His-309 to Ala-314,	Gly-321 to Gln-331	Pro-1 to Asp-10,	Arg-2	Val-65 to Ser-75,	Arg-95 to Asp-104,	Glu-111 to Ile-119,
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Asp-151 to Arg-157.	Lys-1 to Ser-18, Asn-49 to Glu-62	Gln-67 to Ser-76,	Glu-84 to Thr-90,	Thr-104 to Pro-112,	Ser-148 to Arg-156,	Gly-184 to Thr-191,	Pro-203 to Glu-210,	Thr-234 to Ser-240.										-	Lys-1 to Ser-18,	Asn-49 to Glu-62,	Gln-67 to Ser-76,	Glu-84 to Thr-90,	Thr-104 to Pro-112.	Leu-35 to Lys-41,	Leu-61 to Glu-68,	Ser-153 to Gln-158,
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Asn-223 to Pro-228, Ala-259 to Phe-266, Pro-276 to Gly-283, Asp-292 to Phe-307, Ala-318 to Asp-336, Pro-348 to Leu-365, Ala-369 to Thr-393, Gln-398 to Ala-408.	Gly-4 to Ala-19.	Asp-21 to Tyr-27, Pro-66 to Leu-72, Glu-99 to Ala-105, Gly-111 to Val-120, Gln-132 to Ile-138, Asp-152 to Ala-159, Lys-165 to Arg-170, Thr-222 to Cys-229, Arg-265 to Tyr-270, Ser-274 to Asp-283, Asp-299 to Ser-306, Val-316 to Arg-322, Asp-333 to Lys-346, Ser-447 to Arg-452,
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	Ser-9 to Cys-21,	Asn-137 to Leu-142,	Gly-231 to Thr-236,	Arg-284 to Phe-291,	Asn-305 to Asp-313,	Ala-375 to Asn-383,	Cys-404 to Arg-411,	Val-456 to Glu-469,	Glu-516 to Leu-521,	Lys-572 to Tyr-588.	Met-26 to Asn-37,	Glu-42 to Gln-51,	Thr-68 to Ser-95,	Ala-97 to Lys-113,	Asp-156 to Val-161,	Val-208 to Asp-215,	Pro-217 to Ala-228.							-	,		-
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S0031: 2, L0588: 2	S0356: 1, S0354: 1	8: 1, S	93: 1, S	H0431: 1, H0592:	H0643: 1, H0331:	74: 1, H	75: 1, H	0: 1, H	S0049: 1, H0327:	H0012: 1, H0024:	H0014: 1, L0163:	8: 1, S	8: 1, H	32: 1, H	H0038: 1, H0413:	H0059: 1, L0520:	0:1, L	7:1, L	54: 1, L	.0767: 1, L	.0775: 1, L	.0657: 1, L	.0790: 1, L	L0352: 1, H0547: 1	89: 1, H	H0555: 1, L0751:	
S003	S035	S035	H03	H04.	790H	H0574: 1	H0575:]	S0010: 1	S004	H00	H00	S0388: 1	S6028: 1	H0032: 1	H00	H00,	L0770: 1	L077	L0764: 1	L076	L077	L065	L075	L035	90H	H05	L077
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	Leu-33 to Gln-39.	Asn-15 to Glu-24.		-											•				-		•					
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				•														Glu-51 to Phe-60,	Gln-63 to Gly-73,	Thr-85 to Lys-91.	Asp-77 to Lys-82.	:			Pro-1 to Lys-13,	Pro-20 to Lys-39,	Ala-46 to Thr-71,	Pro-112 to Gln-122,
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Gly-129 to Arg-151, Ely-129 to IIe-164, LAIP-188 to Tyr-194, Asn-208 to Pro-217, Sly-237 to Thr-249, Hy-237 to Thr-249, Hy-305 to Ala-285, Ev-292 to Phe-303, Leu-347 to Asn-358, Ser-417 to Asp-330, Phe-449 to Leu-476, Lys-510 to Lys-532, Ser-546 to Glu-562, Lys-570 to Ser-589, Lys-570 to Ser-589, Lys-570 to Ser-589, Lys-570 to Ser-589, Lys-570 to Glu-623.	Glu-5 to Lys-10, Pro-17 to Lys-36, Ala-43 to Thr-68, Pro-109 to Gln-119, Gly-126 to Arg-148, Gly-156 to Ile-161, Ala-185 to Asp-192.	
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Pro-58 to Arg-66,	Lys-95 to Phe-105,	Val-109 to Ala-114.	·									,	,					•		•							
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	Pro-8 to Gly-26, Cys-54 to Cys-66, Gly-73 to His-85.	Ser-31 to Gly-43, Ser-45 to Gly-57.	Ser-37 to Gly-49, Ser-51 to Gly-63, Val-93 to Cys-98.
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	Leu-12 to Ser-19, Glu-108 to Ser-119, Ala-121 to Thr-128, Lys-139 to Ala-149, Arg-153 to Ala-161.	Gln-43 to Ser-49, Ala-60 to Gly-67.	Gln-43 to Ser-49, Ala-60 to Gly-67, Arg-141 to Pro-146.	Trp-14 to Asp-27.	Asp-15 to Leu-21, Ser-59 to His-66, Ile-159 to Tyr-164.
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	Lys-34 to Ala-42, Lys-71 to Leu-76, Arg-188 to Trp-193, Val-215 to Asn-220, Ser-269 to Gln-274, Leu-333 to Lys-341, Thr-354 to Lys-361, Thr-401 to Ile-407, Lys-419 to Arg-427.	Ser-9 to Asn-15, Ser-64 to Gln-69. Cys-1 to Arg-13, Pro-15 to Gly-21, Gly-54 to Ser-59.
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L0774: 6, L0776: 6,	L0748: 6, L0740: 6,	L0752: 6, H0253: 5,	H0181: 5, T0114: 4,	L0750: 4, L0780: 4,	L0755: 4, H0606: 3,	H0087: 3, L0769: 3,	L0764: 3, L0771: 3,	L0806: 3, H0295: 2,	S0354: 2, H0549: 2,	H0298: 2, H0590: 2,	H0510: 2, H0553: 2,	H0038: 2, H0494: 2,	H0509: 2, L0783: 2,	L0809: 2, L0789: 2,	L0665: 2, S0330: 2,	H0696: 2, L0747: 2,	L0596: 2, H0653: 2,	H0661: 1, S0376: 1,	H0282: 1, H0331: 1,	H0574: 1, H0575: 1,	H0251: 1, H0263: 1,	H0204: 1, H0596: 1,	T0110: 1, H0597: 1,	H0327: 1, L0719: 1,	H0544: 1, H0545: 1,	H0178: 1, H0620: 1,	H0375: 1, H0188: 1,
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H0615: 1, H0622: 1, H0633: 1, H0424: 1, H0644: 1, L0640: 1, L0763: 1, L0761: 1, L0642: 1, L0772: 1, L0800: 1, L0642: 1, L0773: 1, L0662: 1, L0773: 1, L0649: 1, L0766: 1, L0669: 1, L0766: 1, L0669: 1, L0766: 1, L0663: 1, L0666: 1, L0663: 1, L0666: 1, L0663: 1, L0743: 1, L0741: 1, L0743: 1, L0731: 1, L0601: 1 and H0423: 1.	AR089: 1, AR061: 0 S0294: 2, H0559: 1 and L0747: 1.	AR089: 3, AR061: 0 S0040: 2, H0547: 2, L0393: 1, H0013: 1, H0427: 1, T0110: 1, T0078: 1, S0364: 1, H0124: 1, H0551: 1,
	Pro-30 to Ala-37, Ala-40 to Arg-49, Ala-152 to Leu-163. Pro-28 to Ala-35, Ala-38 to Arg-47.	Pro-12 to Ala-17, Asp-23 to Phe-28.
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H0100: 1, H0494: 1,	H0509: 1, H0555: 1 and	L0439: 1.	AR061: 0, AR089: 0 H0255: 1, H0305: 1.	S0044: 1, S0037: 1,	S0028: 1 and S0031: 1.	AR061: 1, AR089: 1	H0494: 2, H0544: 1,	S0051: 1, L0754: 1 and	H0542: 1.	,		AR061: 3, AR089: 2	H0618: 3, L0439: 3,	H0124: 2, L0771: 2,	L0766: 2, S0126: 2,	H0445: 2, H0265: 1,	H0253: 1, H0318: 1,	H0421: 1, H0052: 1,	H0197: 1, H0015: 1,	S6028: 1, H0266: 1,	H0380: 1, H0529: 1,	L0803: 1, H0144: 1,	L0352: 1, S0328: 1,	H0539: 1, S0378: 1,	H0134: 1, L0749: 1,	L0777: 1, L0758: 1 and
						Ser-9 to Glu-14,	Arg-22 to Arg-27.			Arg-164 to Arg-169.		Lys-10 to Arg-25,	Glu-40 to Ala-46,	Arg-174 to Ala-181,	Ala-202 to Gln-208.	•								•		
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L0595: 1.	-				AR061: 6, AR089: 4	S0045: 2, S0046: 1,	H0645: 1, H0013: 1,	H0575: 1, H0286: 1,	H0521: 1 and H0136: 1.			AR061: 4, AR089: 4	L0439: 4, L0418: 1,	S0010: 1, L0455: 1,	S0028: 1 and L0741: 1.			,			-				AR061: 8, AR089: 7] H0081: 2, H0549: 1,
	Lys-10 to Arg-25,	Glu-40 to Ala-46,	Arg-174 to Ala-181,	Ala-202 to Gln-208.	Leu-26 to Tyr-32,	Pro-108 to Gln-123.				Leu-26 to Tyr-32,	Pro-108 to Gln-123.	Gly-14 to Glu-32,	Pro-60 to Ala-70,	Thr-145 to Gly-153,	Ser-164 to Leu-169.	8								Phe-4 to Gly-12.	Ser-38 to Asp-46,	Leu-55 to Leu-60,
	209				451					809		452												609	453	,
	3 - 1346		•		3 - 2189	,				3 - 2189		3 - 509								_				473 - 138	601 - 1134	
	283				127				•	284		128			•									285	129	
	971572				1189455				•	952123		951351												956281	1202534	
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H0069: 1. H0046: 1.	H0428: 1, H0553: 1,	H0087: 1, H0529: 1,	L0532: 1, H0521::1 and	H0423: 1.	·			AR061: 1, AR089: 1	L0748: 11, L0749: 6,	L0779: 4, L0438: 2,	H0547: 2, L0747: 2,	L0777: 2, L0596: 2,	H0650: 1, H0013: 1,	H0581: 1, H0046: 1,	H0009: 1, H0266: 1,	H0622: 1, T0042: 1,	S0002: 1, H0695: 1,	H0529: 1, L0762: 1,	L0769: 1, L0771: 1,	L0766: 1, L0376: 1,	L0809: 1, L0666: 1,	L0665: 1, H0658: 1,	H0648: 1, S0044: 1,	H0555: 1, H0187: 1,	L0750: 1, L0752: 1,	L0758: 1, H0343: 1,	50026: 1, 80192: 1,
Lvs-73 to Glu-79.		,			Ser-1 to Trp-6,	Ser-10 to Glu-22,	Pro-112 to Ser-117.	Gln-15 to Asn-20,	Met-59 to Gln-66.		•										-						
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S0194: 1, H0542: 1, H0543: 1 and H0423: 1.		AR089: 10, AR061: 3	H0560: 2, S0342: 1,	H0586: 1, L0471: 1,	H0644: 1, H0617: 1,	H0040: 1, H0641: 1,	H0529: 1, H0519: 1,	S0037: 1 and L0757: 1.	•		AR089: 0	S3010: 2, S0028: 1 and	S0260: 1.			•	-			AR089: 1, AR061: 1	S0028: 3, H0617: 2,	S0045: 1, H0181: 1,	H0383: 1 and S0144: 1.	-
	Gln-15 to Asn-20, Met-59 to Asp-64.	Thr-1 to Asp-7,	Gly-37 to Asn-44,	Arg-175 to Tyr-180,	Lys-190 to Pro-198,	Gln-204 to Leu-209.			Thr-1 to Asp-7,	Gly-37 to Asn-44.	Arg-58 to Glu-63,	Val-80 to Gly-87,	Arg-114 to Lys-119,	Ala-132 to Gly-137,	Val-140 to Asp-145,	Ala-173 to Pro-178.	Ala-25 to Thr-31,	Glu-58 to Arg-63,	Gln-82 to Arg-87.	Arg-1 to Gly-8,	His-33 to Glu-44,	Ala-57 to Gly-62,	Tyr-71 to Arg-77,	Pro-85 to Asn-93,
	611	455							612	•	456						613			457				
	29 - 496	79 - 813	•						089 - 09		835 - 284						82 - 468		•	808 - 2142				
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		-			-		AR061: 3, AR089: 2	L0750: 2, H0024: 1,	H0039: 1, H0622: 1,	H0040: 1 and S0434: 1.	AR061: 1, AR089: 0	L0748: 11, L0749: 6,	L0779: 4, L0438: 2,	H0547: 2, L0747: 2,	L0777: 2, L0596: 2,	H0650: 1, H0013: 1,	H0581: 1, H0046: 1,	H0009: 1, H0266: 1,	H0622: 1, T0042: 1,	S0002: 1, H0695: 1,	H0529: 1; L0762: 1,	L0769: 1, L0771: 1,	L0766: 1, L0376: 1,	L0809: 1, L0666: 1,	L0665: 1, H0658: 1,	H0648: 1, S0044: 1,	H0555: 1, H0187: 1,	
Asp-116 to Ser-122.	Arg-1 to Gly-8,	His-33 to Glu-44,	Ala-57 to Gly-62,	Tyr-71 to Arg-77,	Pro-85 to Asn-93,	Asp-116 to His-121.	Asp-40 to Asn-49,	Cys-65 to Gly-71.	•		Ser-6 to Thr-11.	•									-							
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L0750: 1, L0752: 1, L0758: 1, H0343: 1, S0026: 1, S0192: 1	S0194: 1, H0542: 1, H0543: 1 and H0423: 1	AR089: 40, AR061: 37	S0015: 1 and H0665: 1.		,			AR089: 56, AR061: 55	S0001: 1, S0051: 1 and	S0028: 1.		AR061: 2, AR089: 1	S0278: 1, H0031: 1,	H0617: 1 and S0390: 1.	AR089: 1, AR061: 0	H0013: 1, S0028: 1 and	S0260: 1.			AR089: 5, AR061: 2	S0356: 1, S0354: 1,	S0358: 1, S0376: 1,	H0620: 1, H0023: 1,
		Phe-5 to Val-11,	Ser-28 to Lys-35,	His-119 to Gin-12/.	Phe-5 to Val-11,	Ser-28 to Lys-35,	His-119 to Gin-12/.		·			Ser-19 to Asp-32,	Tyr-58 to Gly-67.		Val-33 to Tyr-44.			Arg-52 to Lys-57,	Glu-67 to Ile-74.	Leu-29 to Pro-47,	Pro-55 to Arg-60,	Pro-99 to Gly-106,	Met-170 to Thr-177,
		460			615			461			919	462			463			617		464			
	-	193 - 723			193 - 723	•		1 - 156			3 - 143	3 - 437	Ü		358 - 2			-221 - 739		<i>L98 - L6</i>	• 401, 2022		
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		126 HKMAC08				,		HSLHS93				HBGOT10		. !	HSDJW73				•	HWMEQ37			
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H0039: 1 and H0593: 1	AR089: 1, AR061: 1 S0050: 1, H0316: 1.	S0428: 1, H0694: 1 and S0031: 1.	,		AR061: 3, AR089: 2 H0542: 2 H0597: 1	H0288: 1, H0124: 1,	H0264: 1, S0344: 1, L0752: 1 and L0581: 1				8, AR061:	S0150: 1				AR089: 1, AR061: 0	H0316: 1	AR061: 3, AR089: 2	L0748: 12, L0749: 7,	.0766: 5, L0803: 4,	.0756: 4, L0769: 3,	.0666: 3, H0547: 3,
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Glu-196 to Ser-207.	Arg-6 to Gly-14, Cvs-20 to Gly-27.	Leu-80 to Pro-86.	Pro-6 to Thr-15,	Asp-27 to Thr-35.	Arg-8 to Arg-14.			Pro-21 to Ser-27,	Arg-42 to Asp-49,	Arg-82 to Ser-90.	Arg-12 to Tyr-23,	Ser-31 to Pro-37,	Thr-42 to Ala-56,	Ile-122 to Lys-128.	Gly-36 to Thr-41.			Pro-1 to Gly-6,	Ile-40 to Lys-46.			
	465		618	•	466			619		•	467				620	468		469				,
	249 - 1685		2 - 292		2 - 646			67 - 372	-		564 - 151				185 - 436	5 - 514		1 - 1236				
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L0777: 3, H0543: 3, S0036: 2, H0040: 2, H0059: 2, L0659: 2, L0790: 2, L0439: 2, H0295: 1, H0657: 1, H0241: 1, S0282: 1, H0228: 1, H0589: 1, S0360: 1, H0431: 1, H0370: 1, H0013: 1, H0156: 1, H0590: 1, H0083: 1, S6028: 1, H0188: 1, T0041: 1, H0188: 1, T0041: 1, L0662: 1, L0643: 1, L0776: 1, L0643: 1, L0776: 1, L0643: 1, L0776: 1, L0740: 1, L0751: 1, L0740: 1, L0755: 1, L0758: 1,		AR089: 2, AR061: 1 L0748: 12, L0749: 7, L0766: 5, L0803: 4,
	Gln-13 to Leu-20, Ala-23 to Leu-29, Lys-58 to Tyr-69.	
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	263 - 613	3 - 338
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L0756: 4, L0769: 3, L0666: 3, H0547: 3, L0777: 3, H0543: 3, S0036: 2, H0040: 2, H0059: 2, L0659: 2, L0790: 2, L0439: 2, H0295: 1, H0657: 1, H0295: 1, H0657: 1, H0228: 1, H0659: 1, H0341: 1, S0282: 1, H0360: 1, H0431: 1, H0156: 1, H0659: 1, H0156: 1, L0643: 1, H0662: 1, L0643: 1, L0776: 1, L0663: 1, L0776: 1, L0663: 1, L0776: 1, L0740: 1, L0751: 1, L0747: 1, L0751: 1, L0747: 1, L0751: 1, H0422: 1 and H0506: 1.	AR061: 2, AR089: 2 H0170: 2 and S0050: 1.	AR089: 1, AR061: 1
	Thr-16 to Lys-25.	Thr-1 to Ile-12,
	471	472
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H0599: 25, L0731: 19,	L0750: 14, L0754: 13,	L0766: 8, L0776: 8,	L0752: 8, L0757: 8,	L0747: 6, L0744: 5,	L0769: 4, L0779: 4,	L0777: 4, S0420: 3,	L0770: 3, L0755: 3,	L0758: 3, L0471: 2,	L0771: 2, L0775: 2,	L0806: 2, L0659: 2,	S0126: 2, H0670: 2,	L0743: 2, L0759: 2,	L0604: 2, H0624: 1,	H0685: 1, H0650: 1,	H0484: 1, H0483: 1,	H0661: 1, S0358: 1,	S0360: 1, S0046: 1,	H0411: 1, H0632: 1,	H0427: 1, S0280: 1,	H0097: 1, H0004: 1,	S0049: 1, H0028: 1,	H0622: 1, L0142: 1,	H0591: 1, L0763: 1,	L0772: 1, L0800: 1,	L0764: 1, L0662: 1,	L0768: 1, L0794: 1,	L0774: 1, L0807: 1,
Pro-78 to Lys-86,	Cys-88 to Leu-97,	Asp-100 to Ile-107,	Pro-176 to Pro-181,	Arg-191 to Met-196,	Pro-200 to Arg-210;	Pro-246 to Ala-259,	Ser-271 to Glu-276,	Asp-298 to Trp-306.																			
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L0809: 1, L0666: 1, L0665: 1, S0148: 1, S0328: 1, S0406: 1, S3014: 1, S0027: 1, S0028: 1, L0599: 1, S0026: 1 and H0667: 1.	-					•		•	AR089: 33, AR061: 10	S0028: 2 and H0178: 1.	AR089: 3, AR061: 1	L0604: 12, S0366: 7,	L0485: 5, H0599: 4,	L0777: 4, H0196: 3,	H0373: 3, L0520: 3,	L0623: 2, S0330: 2,	H0486: 1, H0013: 1,	H0002: 1, H0253: 1,	H0318: 1, L0163: 1,
 *	Pro-1 to Pro-6, Pro-77 to Lys-85,	Lys-8 / to Leu-96, Asp-99 to Ile-106,	Pro-175 to Pro-180,	Arg-190 to Met-195, Pro-199 to Arg-209.	Pro-245 to Ala-258,	Ser-270 to Glu-275,	Asp-297 to Trp-305,	Gly-334 to Ser-339.	Val-11 to Ile-16,	Gly-98 to Pro-103.	Ser-24 to Ser-29.								
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S0364: 1, H0616: 1, H0561: 1 and L0584: 1.			-		,																		AR089: 27, AR061: 12	L0761: 4, H0677: 4,	H0556: 3, H0661: 3,	H0617: 3, H0580: 2,
	Asp-27 to His-32,	Gln-65 to Gly-76,	Lys-80 to Ser-94,	Pro-99 to Asn104,	Gly-126 to Lys-143,	Pro-150 to Lys-156,	Glu-163 to Glu-175,	Val-193 to Asp-204,	Met-230 to Ser-263,	Ala-278 to Gly-291,	Pro-306 to Asn-320,	Asn-328 to Lys-333,	Glu-348 to Glu-355,	Ile-358 to Asn-363,	Glu-375 to Ser-381,	Lys-390 to Arg-395,	Lys-433 to Asn-441,	Ser-456 to Phe-463,	Glu-484 to Lys-490,	Glu-498 to Gly-507,	Glu-535 to Glu-547.				a.	
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H0253: 2, H0135: 2,	H0090: 2, L0509: 2,	L0657: 2, L0438: 2,	S0152: 2, H0436: 2,	H0265: 1, H0161: 1,	H0656: 1, S0420: 1,	S0360: 1, H0550: 1,	H0614: 1, H0250: 1,	H0618: 1, H0544: 1,	H0050: 1, T0010: 1,	H0356: 1, H0252: 1,	H0428: 1, H0040: 1,	L0351: 1, S0344: 1,	S0426: 1, L0499: 1,	L0375: 1, L0776: 1,	L0634: 1, L0809: 1,	L0665: 1, H0144: 1,	H0547: 1, H0658: 1,	S0037: 1, L0744: 1,	L0749: 1, L0777: 1,	H0595: 1, L0366: 1,	H0543: 1, H0422: 1 and	H0506: 1.	AR061: 2, AR089: 1	S0045: 1, S0036: 1,	H0164: 1 and H0026: 1.	
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	AR089: 11, AR061: 4 H0550: 1, S0366: 1 and H0134: 1	AR089: 6, AR061: 1	H0478: 3, S0278: 2,	L0731: 2, S0001: 1,	S0360: 1, S0132: 1,	H0619: 1, H0263: 1,	S0036: 1, H0040: 1,	H0494: 1, S0142: 1,	S0344: 1, L0764: 1,	L0766: 1, S3014: 1,	L0748: 1, H0445: 1 and	S0434: 1.			-		-					AR061: 6, AR089: 3	H0623: 3, H0620: 2,	H0521: 2, H0542: 2,	H0556: 1, H0341: 1,
Asn-95 to Ser-100.	His-1 to Asp-6, Pro-63 to Leu-74.	Asp-1 to Lys-7,	Gly-27 to Gln-32,	Arg-67 to Gly-77.		-						-	-							44 A		Asn-60 to Gln-74,	Pro-97 to Arg-103,	Pro-128 to Gln-134,	Ser-141 to Glu-154,
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Ala-157 to Arg-163.			-					•	Asn-18 to Gly-25,	Lys-33 to Ser-43,	His-54 to Cys-63,	Ser-71 to Gly-76,	Ser-85 to Gln-93.	2				- 30		. •					
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	Gln-2 to Ser-11,	Pro-52 to Gly-61,	Thr-68 to Gly-103, Lys-114 to Ala-120,	Pro-122 to Arg-127,	Gly-136 to Thr-147,	Asn-150 to Arg-167.	Gly-1 to Ser-11,	Ser-18 to Ala-25,	Ser-70 to Cys-77,	Asp-89 to His-104.	Ser-65 to Cys-72,	Asp-84 to His-99,	Arg-107 to Asn-112.	Ile-45 to Arg-52,	Phe-77 to Pro-85.			-		-		
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Leu-56 to Trp-62, Trp-88 to Pro-98, Cys-118 to Cys-128, Lys-207 to Thr-213, Ile-224 to Ser-233, Gly-254 to Ile-261, Gln-268 to Asp-274.				·	
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	His-1 to Asp-7, Asp-56 to Tyr-64.	Pro-1 to Asp-23, Ile-55 to Gly-81, Glu-150 to Glu-155, Gly-194 to Gly-200.
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	Gly-1 to Ala-6, Ser-19 to Ser-27,	Phe-31 to Leu-55,	Glu-72 to His-79,	Asn-120 to Gly-126,	Arg-138 to Arg-163.	Ser-9 to Ser-17,	Phe-21 to Leu-45.												,	-				
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Arg-31 to Val-38,	Arg-80 to Pro-90,	Asn-183 to Val-190,	Val-318 to Leu-327,	Leu-329 to Leu-354,	Gln-357 to Glu-367,	Leu-373 to Glu-380,	Arg-391 to Gly-396,	Ser-444 to Ser-457.	Arg-31 to Val-38,	Arg-80 to Pro-90.						Met-1 to Gly-17,	Pro-22 to Gly-30,	Gly-72 to His-82,	Leu-89 to Lys-95.				Asn-31 to Leu-38,	Cys-53 to Cys-64,	Gly-139 to Cys-144.	
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	Gly-1 to Pro-11, Ser-39 to Thr-53.	Met-77 to Asn-92.	Gly-12 to Gly-20, Ser-86 to Glu-94, Pro-103 to Pro-110.
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			Thr-3 to Gln-9,	Phe-36 to Ala-41,	His-52 to Ala-63,	Ala-81 to Ser-100,	Pro-122 to Ser-134.									Gly-58 to Cys-64,	Lys-74 to Gln-81,	Thr-90 to Asp-99,	Met-113 to Ser-118,	Met-144 to Gln-150,	Gln-166 to Gly-173,	Thr-180 to Leu-187,	Ser-246 to Asp-256.	•				
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130	L0766: 16, L0749: 13,	L0748: 12, L0777: 9,	L0740: 6, H0341: 3,	H0144: 3, H0268: 2,	L0779: 2, L0780: 2,	H0624: 1, S0430: 1,	H0661: 1, S0420: 1,	S0045: 1, S0046: 1,	S0222: 1, H0013: 1,	H0544: 1, L0157: 1,	H0320: 1, H0428: 1,	H0040: 1, H0551: 1,	H0412: 1, H0623: 1,	L0564: 1, H0560: 1,	H0646: 1, L0520: 1,	L0769: 1, L0772: 1,	L0364: 1, L0803: 1,	L0650: 1, L0378: 1,	L0791: 1, L0666: 1,	H0519: 1, H0539: 1,	S0152: 1, H0521: 1,	S0044: 1, H0436: 1,	L0754: 1, L0747: 1,	L0750: 1, L0731: 1,	L0758: 1, L0589: 1,	L0608: 1, L0366: 1,	S0192: 1 and H0543: 1.
Ser-70 to Asp-77,	Ser-85 to Asp-90,	Asp-139 to Gly-145,	Ile-207 to Asp-213,	Arg-229 to Met-234,	Gly-259 to Ser-264,	Ile-281 to Ser-288,	Asp-337 to Leu-343,	Gln-369 to Ile-376,	Gly-429 to Ser-440,	Gln-448 to Val-456,	Gln-461 to Thr-474.			·											-		
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	AR061: 8, AR089: 4 L0759: 3, S0152: 2, L0749: 2, H0441: 1, H0013: 1, H0427: 1, H0156: 1, H0318: 1, H0597: 1, H0050: 1, S0386: 1, H0538: 1, L0803: 1 and L0809: 1.		AR089: 2, AR061: 1 H0014: 1, H0039: 1,	30380; 1 and L0740; 1.		·	AR089: 5, AR061: 3	L0747: 12, L0766: 10,	H0683: 9, L0776: 7,	H0521: 6, L0764: 4,
Ala-38 to Thr-45, Ser-70 to Asp-77, Ser-85 to Asp-90, Asp-139 to Gly-145, Arg-189 to Asp-196.			Asn-16 to Ser-23, Lys-53 to Val-61,	Leu-116 to Ala-121, Gln-152 to I vs-168	Arg-178 to Lys-183, Asp-196 to Glu-203, Glu-220 to Ser-233.	Asn-16 to Ser-23, Lys-53 to Asp-60.	Leu-27 to Pro-34,	Pro-40 to Lys-51,	Asn-85 to Phe-90,	Arg-102 to Leu-140,
652	543	653	45			654	545			
18 - 644	159 - 683	159 - 563	161 - 886			226 - 861	1 - 780			
328	219	329	077			330	221			
911607	1153892	766126	5/27511	-		966135	974684			
	HHFGD38		HVAOGII				HUVDR03			
			210				211			

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									,																		
L0439: 4, L0731: 4,	H0624: 3, S0222: 3,	H0457: 3, H0051: 3,	L0770: 3, L0769: 3,	L0790: 3, L0666: 3,	L0664: 3, H0547: 3,	L0750: 3, L0757: 3,	L0759: 3, H0050: 2,	H0056: 2, S0210: 2,	L0662: 2, L0774: 2,	L0519: 2, L0665: 2,	H0519: 2, L0748: 2,	L0751: 2, S0242: 2,	H0556: 1, H0657: 1,	H0341: 1, H0484: 1,	H0125: 1, S0418: 1,	S0354: 1, S0300: 1,	S0278: 1, H0370: 1,	H0392: 1, H0438: 1,	H0600: 1, H0592: 1,	T0039: 1, H0250: 1,	H0427: 1, H0042: 1,	H0575: 1, H0004: 1,	H0581: 1, H0421: 1,	H0012: 1, H0083: 1,	H0408: 1, H0355: 1,	H0266: 1, H0271: 1,	H0622: 1, H0169: 1,
Gly-145 to Asp-191,	Glu-219 to His-227.					*											-										
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H0135: 1, H0264: 1, H0272: 1, H0488: 1, H0412: 1, H0623: 1, H0059: 1, H0625: 1, H0641: 1, S0426: 1, L0761: 1, L0646: 1, L0657: 1, L0803: 1, L0657: 1, L0803: 1, H0701: 1, S0428: 1, H0701: 1, S0428: 1, H0701: 1, S048: 1, H0659: 1, H0648: 1, H0672: 1, S0328: 1, L0744: 1, L0754: 1, L0756: 1, L0779: 1, L0756: 1, L0779: 1, H0136: 1, S0192: 1, H0543: 1, H0422: 1 and S0412: 1.	AR089: 13, AR061: 4 L0750: 2, H0370: 1, H0494: 1 and S0042: 1.	AR061: 1, AR089: 1 S0048: 1 and T0010: 1.
H0135: 1 H0272: 1 H0412: 1 H0641: 1 L0761: 1 L0657: 1 L0663: 1 H0670: 1 H0659: 1 H0672: 1 L0744: 1 L0752: 1 L0752: 1 L0752: 1 H0631: 1 S0380: 1,	L0750	R061 S0048
<u> </u>	V ⊞	V
		His-3 to Leu-15, Tyr-28 to Ala-34, Gly-52 to Glu-57,
	546	547
	58 - 288	1968 - 229
	222	223
	689811	954681
	HUDAE29	HIBCJ89
	212	213

					·
		AR089: 1, AR061: 1 L0439: 4, T0010: 2 and H0038: 1.	AR089: 14, AR061: 6 H0693: 44, L0604: 5, S0366: 4, L0805: 4, H0637: 3, L0766: 3, H0672: 3, H0549: 2, H0671: 2, L0777: 2, H0661: 1, H0580: 1, H0428: 1, S0364: 1, L0641: 1, L0644: 1, L0655: 1, L0809: 1, L0791: 1, L0666: 1, L0653: 1, T0068: 1, H0576: 1, L0780: 1, L0731: 1, L0584: 1 and H0576: 1, L0780: 1,		AR089: 14, AR061: 8 L0766: 3, L0805: 3, L0659: 3, L0744: 3, L0794: 2, L0776: 2, L0665: 2, H0648: 2,
Ser-123 to Gly-136.		Phe-15 to Glu-24.	Pro-26 to Ala-38, Lys-85 to Gly-97, Tyr-120 to Glu-131, Asp-158 to Leu-168, Asn-187 to Gly-197, Ser-204 to Asp-209.	Lys-51 to Gly-63, Tyr-86 to Glu-97.	Thr-1 to Arg-7, Asn-34 to Gly-41, Thr-67 to Asn-75.
	929	548	549	959	550
	367 - 1713	29 - 403	3 - 1784	103 - 591	3 - 317
	331	224	225	332	226
	963279	504158	1195806	702070	1150878
		HIBEG40	HWBEG33	·	HWHKD22
		214	215		216

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H0305: 1, H0586: 1, H0599: 1, H0428: 1, H0551: 1, L0763: 1, L0637: 1, L0862: 1, L0768: 1, L0806: 1, L0804: 1, L0806: 1, L0655: 1, L0661: 1, L0787: 1, S0374: 1, H0520: 1, L0740: 1, L0770: 1, L0756: 1, L0777: 1, L0752: 1,	·	AR089: 1, AR061: 0 S0052: 1 and S0028: 1.	AR061: 1, AR089: 1	L0/76: 20, L0/77: 9, L0439: 6, L0438: 4,	L0752: 4, L0591: 4,	H0013: 3, H0052: 2, H0024: 2 1 0415: 1	S0212: 1, S0360: 1,	H0586: 1, H0596: 1,	H0050: 1, S0050: 1,	H0373: 1, S0051: 1,	S6028: 1, H0188: 1,	S0386: 1, S0448: 1,
	Asn-48 to Gly-55, Thr-81 to Asn-89.		Ser-40 to Tyr-45,	Ala-61 to Pro-/1, Gly-92 to Asp-98,	Ala-145 to Asp-151,	Pro-197 to Cys-205, I en-224 to Glv-235	Glu-241 to Ala-254,	Ser-256 to Asn-262,	Asp-279 to Glu-290,	Ser-296 to Gly-303,	Lys-340 to Arg-345,	Ile-347 to Tyr-354.
		551	552									
	152 - 508	1 - 282	1 - 1083									
	333	227	228		٠							
	963626	765497	944511									
		HSLF041	HE9SE46								•	
		217	218			·						

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	-7 (8.1			 -				•;				•							_							
50306.1 1.0369.1	L0774: 1, L0775: 1,	L0805: 1, H0144: 1,	T0068: 1, S0330: 1,	L0745: 1, L0750: 1,	L0779: 1, L0755: 1,	L0731: 1, S0260: 1,	L0596: 1, L0608: 1 and	H0665: 1.	AR089: 18, AR061: 18	H0618: 1, H0253: 1,	H0012: 1, H0620: 1,	H0181: 1 and H0617: 1.	AR089: 1, AR061: 1		-											
									Thr-1 to Leu-7.		*	;	·	Met-1 to Lys-11,	Asp-96 to Ile-104,	Asn-127 to Ser-140,	Gln-185 to Arg-190,	Lys-221 to Ser-231,	Ala-254 to Val-262,	His-295 to Asp-300,	Leu-304 to Ser-323,	Ser-327 to Gln-333,	Ala-345 to Ser-354,	Ala-370 to Ser-384,	Thr-396 to Gly-402,	Leu-413 to Pro-423,
									553		•		554	959												
	7	~			· · · · · · ·			,	216 - 836				17 - 2389	144 - 2336												
				v				•	229				230	334				-								,
,					•				864276				1227138	1056330			•					•				•
								,	HTLDW37				HWAFG54 1227138									•	•			
					•				219	,			220	 						- 1						

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	-		103850, 114835, 116800, 140100, 192090, 192090,
i)			16q22.1
	AR089: 31, AR061: 25	AR061: 7, AR089: 4 S0116: 2, H0510: 2, H0144: 2, H0521: 2, L0748: 2, H0556: 1, T0049: 1, H0580: 1, H0393: 1, H0587: 1, H0651: 1, H0375: 1, H0646: 1, S0002: 1, L0752: 1 and L0731: 1.	AR061: 1, AR089: 1 H0457: 8, H0255: 6, L0743: 4, H0650: 2, S0354: 2, H0581: 2, L0747: 2, H0341: 1, S0376: 1, H0580: 1, H0069: 1, H0042: 1,
Gly-432 to Val-438, Ser-478 to Phe-485, Arg-487 to Lys-506, Ser-528 to Ser-547, Asn-557 to Ala-566, Asp-586 to Glu-597, Glu-644 to Pro-656, Leu-663 to Arg-671, Ser-700 to Asp-707.	· .		Arg-50 to Gln-56, Gly-109 to Glu-119, Gln-131 to Asp-137, Gly-149 to Gly-159, Leu-184 to Glu-218, Val-239 to Ile-245.
	555	556	557
-	3 - 410	3 - 752	1 - 1302
ž	231	232	233
	810433	921175	932448
-	HKAFS73	HTXJD74	HSIGQ50
	221	222	223

192090, 192090, 245900, 245900, 276600, 600223		
H0036: 1, H0590: 1, H0251: 1, H0085: 1, H0123: 1, H0687: 1, H0213: 1, H0135: 1, H0040: 1, H0646: 1, S0002: 1, H0593: 1, H0555: 1, L0748: 1, L0731: 1, L0758: 1, L0796: 1, H0543: 1 and H0506: 1.	AR089: 1, AR061: 1 H0657: 1, S0376: 1, H0123: 1, H0428: 1, L0646: 1, L0662: 1, L0803: 1, L0659: 1, L0790: 1, L0791: 1, H0660: 1 and L0759: 1.	AR089: 34, AR061: 19 L0664: 2, H0483: 1, S0376: 1, L0762: 1, L0657: 1, L0771: 1, L0665: 1, H0658: 1, H0670: 1 and L0779: 1.
	Gly-40 to Gly-46, Gln-60 to Arg-69, Lys-84 to Trp-91, Leu-112 to Arg-118.	Ser-15 to Tyr-24, Met-47 to Tyr-56, Gly-127 to Ser-133.
·		559
	3 - 647	3 - 677
	234	235
	932607	971537
	HWWDY45	HNSMB24
	224	225

·····						
9	•					
AR089: 3, AR061: 2 6	L0774: 3, L0771: 2,	L0766: 2, L0779: 2,	S0376: 1, L0646: 1,	L0764: 1, L0666: 1,	L0748: 1, L0731: 1,	L0593: 1 and H0423: 1.
	•					
095					•	٠
691 - 662		7	٠			
236					·	
946862	•					
226 HWLOU63 946862		•				
226				-		

The first column in Table 1A provides the gene number in the application corresponding to the clone identifier. The second column in Table 1A provides a unique "Clone ID NO:Z" for a cDNA clone related to each contig sequence disclosed in Table 1A. This clone ID references the cDNA clone which contains at least the 5' most sequence of the assembled contig and at least a portion of SEQ ID NO:X was determined by directly sequencing the referenced clone. The reference clone may have more sequence than described in the sequence listing or the clone may have less. In the vast majority of cases, however, the clone is believed to encode a full-length polypeptide. In the case where a clone is not full-length, a full-length cDNA can be obtained by methods described elsewhere herein.

- [36] The third column in Table 1A provides a unique "Contig ID" identification for each contig sequence. The fourth column provides the "SEQ ID NO:" identifier for each of the contig polynucleotide sequences disclosed in Table 1A. The fifth column, "ORF (From-To)", provides the location (i.e., nucleotide position numbers) within the polynucleotide sequence "SEQ ID NO:X" that delineate the preferred open reading frame (ORF) shown in the sequence listing and referenced in Table 1A, column 6, as SEQ ID NO:Y. Where the nucleotide position number "To" is lower than the nucleotide position number "From", the preferred ORF is the reverse complement of the referenced polynucleotide sequence.
- [37] The sixth column in Table 1A provides the corresponding SEQ ID NO:Y for the polypeptide sequence encoded by the preferred ORF delineated in column 5. In one embodiment, the invention provides an amino acid sequence comprising, or alternatively consisting of, a polypeptide encoded by the portion of SEQ ID NO:X delineated by "ORF (From-To)". Also provided are polynucleotides encoding such amino acid sequences and the complementary strand thereto.
- Column 7 in Table 1A lists residues comprising epitopes contained in the polypeptides encoded by the preferred ORF (SEQ ID NO:Y), as predicted using the algorithm of Jameson and Wolf, (1988) Comp. Appl. Biosci. 4:181-186. The Jameson-Wolf antigenic analysis was performed using the computer program PROTEAN (Version 3.11 for the Power MacIntosh, DNASTAR, Inc., 1228 South Park Street Madison, WI). In specific embodiments, polypeptides of the invention comprise, or alternatively consist of, at least one, two, three, four, five or more of the predicted epitopes as described in Table 1A. It will be appreciated that depending on the analytical criteria used to predict antigenic determinants, the exact address of the determinant may vary slightly.

[39] Column 8 in Table 1A provides an expression profile and library code: count for each of the contig sequences (SEQ ID NO:X) disclosed in Table 1A, which can routinely be combined with the information provided in Table 4 and used to determine the tissues, cells, and/or cell line libraries which predominantly express the polynucleotides of the invention. The first number in column 8 (preceding the colon), represents the tissue/cell source identifier code corresponding to the code and description provided in Table 4. For those identifier codes in which the first two letters are not "AR", the second number in column 8 (following the colon) represents the number of times a sequence corresponding to the reference polynucleotide sequence was identified in the tissue/cell source. Those tissue/cell source identifier codes in which the first two letters are "AR" designate information generated using DNA array technology. Utilizing this technology, cDNAs were amplified by PCR and then transferred, in duplicate, onto the array. Gene expression was assayed through hybridization of first strand cDNA probes to the DNA array, cDNA probes were generated from total RNA extracted from a variety of different tissues and cell lines. Probe synthesis was performed in the presence of ³³P dCTP, using oligo(dT) to prime reverse transcription. After hybridization, high stringency washing conditions were employed to remove nonspecific hybrids from the array. The remaining signal, emanating from each gene target, was measured using a Phosphorimager. Gene expression was reported as Phosphor Stimulating Luminescence (PSL) which reflects the level of phosphor signal generated from the probe hybridized to each of the gene targets represented on the array. A local background signal subtraction was performed before the total signal generated from each array was used to normalize gene expression between the different hybridizations. The value presented after "[array code]:" represents the mean of the duplicate values, following background subtraction and probe normalization. One of skill in the art could routinely use this information to identify normal and/or diseased tissue(s) which show a predominant expression pattern of the corresponding polynucleotide of the invention or to identify polynucleotides which show predominant and/or specific tissue and/or cell expression.

[40] Column 9 in Table 1A provides a chromosomal map location for certain polynucleotides of the invention. Chromosomal location was determined by finding exact matches to EST and cDNA sequences contained in the NCBI (National Center for Biotechnology Information) UniGene database. Each sequence in the UniGene database is assigned to a "cluster"; all of the ESTs, cDNAs, and STSs in a cluster are believed to be derived from a single gene. Chromosomal mapping data is often available for one or more

sequence(s) in a UniGene cluster; this data (if consistent) is then applied to the cluster as a whole. Thus, it is possible to infer the chromosomal location of a new polynucleotide sequence by determining its identity with a mapped UniGene cluster.

[41] A modified version of the computer program BLASTN (Altshul et al., J. Mol. Biol. 215:403-410 (1990); and Gish and States, Nat. Genet. 3:266-272 (1993)) was used to search the UniGene database for EST or cDNA sequences that contain exact or near-exact matches to a polynucleotide sequence of the invention (the 'Query'). A sequence from the UniGene database (the 'Subject') was said to be an exact match if it contained a segment of 50 nucleotides in length such that 48 of those nucleotides were in the same order as found in the Query sequence. If all of the matches that met this criteria were in the same UniGene cluster, and mapping data was available for this cluster, it is indicated in Table 1A under the heading "Cytologic Band". Where a cluster had been further localized to a distinct cytologic band, that band is disclosed; where no banding information was available, but the gene had been localized to a single chromosome, the chromosome is disclosed.

Once a presumptive chromosomal location was determined for a polynucleotide of the invention, an associated disease locus was identified by comparison with a database of diseases which have been experimentally associated with genetic loci. The database used was the Morbid Map, derived from OMIMTM (*supra*). If the putative chromosomal location of a polynucleotide of the invention (Query sequence) was associated with a disease in the Morbid Map database, an OMIM reference identification number was noted in column 10, Table 1A, labelled "OMIM Disease Reference(s)". Table 5 is a key to the OMIM reference identification numbers (column 1), and provides a description of the associated disease in Column 2.

TABLE 1B

Clone ID NO:Z	SEQ ID NO:X	CONTIG ID:	BAC ID: A	SEQ ID NO:B	EXON From-To
HE2KJ64	12	906019	AC020570	659	1-67
111121304	12	300013	AC020370	039	406-542
					1507-1624
					2333-2429
					4080-4222
	:				4398-4455
					4561-4630
					4836-4971
					7386-7427
					7521-7596
HE2KJ64	12	906019	AC020570	660	1-247
HLICC37	14	856958	AL365356	661	1-195
					1135-2232
					2239-3110
HLICC37	14	856958	AL365356	662	1-173
HLICC37	14	856958	AL365356	663	1-141
HLTER04	23	590990	AC018845	664	1-273
				,	320-800
					866-1324
					1551-2419
					3945-4348
					5055-5373
00					5597-5685
	•				6123-6519
					7020-7482
					7751-7856
					8955-9162
					9398-9496
					10809-11159
					13498-13544
					13809-14276
					14343-14490
					14632-14762
					16544-18402
HLTER04	23	590990	AC007338	665	1-273
					320-801
					867-1325
					1552-2420

1 1					
					3946-4349
					5056-5374
					5598-5686
					6124-6520
					7021-7483
					7752-7857
					8956-9163
					9399-9497
					10810-11160
					13499-13545
1					13810-14277
					14344-14491
					14633-14763
		-			16545-18403
HLTER04	23	590990	AC018845	666	1-249
HLTER04	23	590990	AC007338	667	1-249
H2MBY83	25	752124	AC017104	668	1-540
H2MBY83	25	752124	AC017104	669	1-548
HMZAD58	27	975304	AC078916	670	1-364
HMZAD58	27	975304	AC022305	671	1-686
HMZAD58	27	975304	AC002518	672	1-247
HMZAD58	27	975304	AC072032	673	1-364
HMZAD58	27	975304	AC078916	674	1-288
HMZAD58	27	975304	AC072032	675	1-288
HCHNH17	28	975378	AC026236	676	1-141
HBIMF04	36	951601	AL022328	677	1-103
					1215-1770
					2471-2545
					3028-3108
					3680-3960
					4352-4494
					4925-5476
					6623-6828
					6888-9053
					9409-10241
HBIMF04	36	951601	AL022328	678	1-333
HBIMF04	36	951601	AL022328	679	1-186
					376-570
					1511-2312
					2355-2630
					2996-3446

			,		3617-4004
					4225-5042
					5275-5664
					5695-5783
					6915-7130
	•				7265-7787
					8377-9065
					9159-9294
					9608-9952
					10071-10419
					11431-11799
					12322-12621
1					12641-12911
					14491-14580
					14653-14848
		-	:		15670-15856
					15949-16109
			1		16183-16596
HOCQD08	39	972981	AC018568	680	1-1718
HOCQD08	39	972981	AC018568	681	1-425
HE8DL23	43	693641	AL135999	682	1-63
					405-942
					1196-1502
					2152-6417
8					6659-6755
					7033-7385
					7481-7535
					7647-8163
					8230-8492
			ļ		8590-9909
					10114-10360
					10420-10783
					10970-11960
					12018-13492
					14130-14528
					14563-15789
HE8DL23	43	693641	AL135999	683	1-410
HAJBU67	55	856922	AC008910	684	1-1685
					1960-2928
HAJBU67	55	856922	AC026230	685	1-1686
		1000			1961-2933
			1	1	1 1701 2733

HAJBU67	55	856922	AC008910	686	1-326
HAJBU67	55	856922	AC026230	687	1-91
HAJBU67	55	856922	AC026230	688	1-325
HCEMY90	68	932927	AC024242	689	1-274
					1243-1357
					1994-2270
HCEMY90	68	932927	AF214633	690	1-274
					1243-1357
					1994-2270
HCEMY90	68	932927	AC024242	691	1-232
HCEMY90	68	932927	AF214633	692	1-130
HHFLF63	69	933854	AC023295	693	1-75
					1512-1564
HDTDG41	72	942490	AL137848	694	1-175
					2422-2550
					3441-3583
					4018-4129
					8219-8689
					9767-9876
	:				11592-11892
					14228-14324
					15025-15162
					16319-16590
					17309-18595
HDTDG41	72	942490	AL137848	695	1-196
HFEBN52	82	810429	AL136001	696	1-61
					290-371
į					654-779
					2128-2223
ĺ		ĺ			2337-2372
					2507-2674
					3747-4249
					4554-4644
					5223-5557
					5604-5916
				1	6827-6930
					6949-7329
					7852-8047
HFEBN52	82	810429	AL359399	697	1-61
					290-371
	J				654-779

					2128-2223
					2337-2372
					2507-2674
					3747-4249
					4554-4644
					5221-5555
					5602-5914
			:		6825-6928
					6947-7327
					7850-8045
HFEBN52	82	810429	AL136001	698	1-430
HFEBN52	82	810429	AL359399	699	1-430
HAJBH69	99	812164	AL035496	700	1-565
				1	855-1099
					2067-4150
,					4159-4449
					4474-4747
					5104-5234
					5852-5937
					6421-6561
).	Ì		7510-7799
					8583-9223
]			9477-9989
					10109-10208
					11605-12056
					12474-12574
					13276-13359
					14559-14890
					14968-16129
					16629-16740
					16984-17214
					17460-17816
HMAER78	102	702735	AC074333	701	1-357
НТЕРМ33	105	870561	AL132776	702	1-42
					1020-1195
					2173-2338
					6839-7029
					11880-12103
НТЕРМ33	105	870561	AL132776	703	1-173
НТЕРМ33	105	870561	AL132776	704	1-791
HDTEJ81	107	919873	AC004707	705	1-74

		1		T	
					285-478
					553-872
					2612-4708
					4745-5348
HDTEJ81	107	919873	AC004707	706	1-318
					1694-1796
					2541-2601
					2726-3334
					4150-4509
	:				4632-4791
					5026-5134
					8019-8346
					8944-9470
					12238-12412
					14290-16770
					17028-17771
					19503-19606
					21647-22467
HCGMG56	118	953660	AC004707	707	1-604
					641-2737
			j		4477-4796
					4871-5064
	111113				5275-5348
HCGMG56	118	953660	AC004707	708	1-821
					2862-2965
					4697-5440
					5698-8178
					10056-10230
	-00				12998-13524
					14122-14449
					17334-17442
					17677-17836
					17959-18318
					19134-19742
			j		19867-19927
					20672-20774
					22150-22467
HE8MI76	123	911474	AL137008	709	1-97
					446-576
			}		761-1233
					3775-3946

			,		r
					4867-5024
					5520-5729
				-	8345-8467
					10681-10858
	ļ				11553-11879
					12483-14416
					14439-14940
					15077-15549
	·				15779-15907
					20468-20613
					21617-21807
					23498-23598
					23636-23733
					23851-24271
					25734-26340
1					26686-26850
*					27674-27830
					28001-28075
· ,					29807-30301
					30480-31201
					31218-31488
					31758-31878
				į.	32812-33412
					33772-34391
					34798-34911
					36778-37158
					37234-37825
					38688-39969
HNSAB28	154	881286	AC010188	710	1-151
					1103-1517
					2286-2664
					4067-4735
					4740-4859
					5876-6449
					7178-7278
					7318-7451
					7539-7983
					8131-8235
					8418-9210
					9619-9776
					11087-12216

HNSAB28	154	881286	AC010188	711	1-420
					442-1482
HTTEP70	155	917729	AC005546	712	1-84
					94-607
					687-742
					971-1123
				}	1271-1463
					2970-3130
					3726-3851
					3920-4035
					4307-4724
					5193-5352
					6432-6975
					7007-7190
					7271-7363
-					7504-7738
		,			7747-7841
					8468-8620
					8879-8995
					9088-9166
				ľ	9632-9736
					9743-9875
					9953-10058
					10840-10955
				-	11128-11473
					11656-11837
HTTEP70	155	917729	AC005546	713	1-74
HUSGZ51	162	955542	AC018568	714	1-1718
HUSGZ51	162	955542	AC018568	715	1-425
HNFFR23	165	585289	AC008751	716	1-343
HFOZC96	167	926685	AF238376	717	1-145
					304-383
					2385-2851
					3341-3588
					4343-4428
					4631-4797
					6602-6724
					7496-8173
					8368-9341
HHERB37	169	708477	AL355377	718	1-505
					662-2071

HKAED89	178	827573	AF038458	719	1-630
					1311-1416
					2481-4022
					4952-5252
			i		6370-6479
			,		7623-8269
HWWGT02	185	908017	AC004188	720	1-150
					500-1073
					1818-2402
					2467-3243
			10000000		3940-4026
HWWGT02	185	908017	AB014086	721	1-150
					500-1072
					1817-2401
ļ					2466-3242
					3939-4025
HWWGT02	185	908017	AC004188	722	1-699
HWWGT02	185	908017	AB014086	723	1-699
HODFI03	187	918008	AC007041	724	1-402
	1				3126-3268
					3901-4312
					4472-5358
					6517-6670
		}			6767-7912
					8251-8380
					8609-8730
					9249-9427
					9575-10072
					10942-11345
		ļ			11359-11545
					11877-14991
HODFI03	187	918008	AC007041	725	1-322
HODFI03	187	918008	AC007041	726	1-381
HWHHR02	188	919169	AF053356	727	1-71
					234-304
					320-826
					878-1099
			8		1233-1683
1					2077-3297
HWHHR02	188	919169	AF053356	728	1-434
HWHHR02	188	919169	AF053356	729	1-152

			1		
HSVBQ03	189	924850	AC004477	730	1-326
				}	1029-1626
					2309-2345
					2958-3015
					3982-4124
:					5005-5248
					5482-6071
					6519-6577
					7045-7136
					7692-7780
					8037-8184
					9575-9866
					10372-10789
					11501-11618
HE9GZ52	192	964579	AL359881	731	1-183
					574-652
					897-1212
	<u> </u>				1599-1902
HE9GZ52	192	964579	Z98884	732	1-569
					4314-4514
	,				4905-4983
					5228-5543
		ļ			5930-6233
HE9GZ52	192	964579	Z98884	733	1-298
HSDJH63	195	941120	AC012224	734	1-1316
HSDJH63	195	941120	AC044892	735	1-1394
HSDJH63	195	941120	AC006252	736	1-1394
HSDJH63	195	941120	AC006252	737	1-111
					281-410
					1326-1983
HWDAE40	199	947007	AC016605	738	1-2114
HWDAE40	199	947007	AC008917	739	1-107
					510-2620
HWDAE40	199	947007	AC008917	740	1-426
HUVHH77	200	948377	AL132641	741	1-2545
HUVHH77	200	948377	AL132641	742	1-4063
					4990-5958
HUVHH77	200	948377	AL132641	743	1-775
HTLIT03	201	966870	AC009077	744	1-89
					471-679
					809-978

					3619-4024
					5223-5374
	Ì				6500-6876
					7519-7607
					8279-8386
					8536-9192
HTLIT03	201	966870	AC004531	745	1-84
					643-971
					1003-1047
					2794-2855
					7497-7541
					8459-8546
					8885-9170
					12745-12811
					12995-13065
				ļ	14987-15122
					16524-16612
					16994-17202
		[17332-17501
					20142-20547
]	21746-21897
				,	23023-23399
					24802-24909
					25059-25715
HTLIT03	201	966870	AC009077	746	1-139
HTLIT03	201	966870	AC009077	747	1-114
HTLIT03	201	966870	AC004531	748	1-108
HRABP94	209	970481	AL136222	749	1-73
					226-264
					289-1812
[1968-2177
					2300-2813
					2951-3091
					3146-3222
					3597-3888
HRABP94	209	970481	AL109947	750	1-35
}					90-146
					603-976
					1504-1816
					1908-2118
					2389-2496

					3139-4163
					5195-5455
					5670-5784
					5971-6356
					6875-7024
					7362-8082
					8097-9620
				[9776-9985
					10108-10621
1					10759-10899
					10954-11030
					11405-11696
HRABP94	209	970481	AL359711	751	1-35
					90-146
		j			603-976
	0				1504-1816
					1908-2118
					2389-2496
					3139-4163
					5195-5455
					5670-5784
_					5971-6356
					6875-7024
					7362-8082
1					8097-9620
					9776-9985
	÷			•	10108-10621
					10759-10899
					10954-11030
					11405-11696
HRABP94	209	970481	AL136222	752	1-479
		}			502-655
					841-948
					1038-1393
]			1624-1713
		1			1856-1951
}					2057-2373
					2467-2567
					2696-3160
HRABP94	209	970481	AL109947	753	1-479
					502-655

					841-948
					1038-1393
					1624-1713
					1856-1951
Ì					2057-2373
					2467-2567
					2696-3160
HRABP94	209	970481	AL359711	754	1-479
					502-655
					841-948
					1038-1393
					1624-1713
					1856-1951
					2057-2373
					2467-2567
					2696-3160
HRABP94	209	970481	AL109947	755	1-532
HRABP94	209	970481	AL359711	756	1-532
HTTHF21	213	921596	AC013264	757	1-1071
					3263-3406
					4512-4719
HTTHF21	213	921596	AC074092	758	1-1071
HTTHF21	213	921596	AC013264	759	1-527
HTTHF21	213	921596	AC074092	760	1-527
HJMBN52	215	966226	AF276758	761	1-184
					756-1081
					1289-1644
					3033-3467
HJMBN52	215	966226	AC024049	762	1-184
					755-1080
					1283-1637
					3026-3458
HSIGQ50	233	932448	AC015551	763	1-250
			,		418-479
					572-642
					1076-1152
					2851-2927
					3010-3133
					3242-3338
					3438-3518
					3612-3715

					3840-3987
					4189-4308
					4594-4869
					4912-5046
					5149-5298
					5473-6592
					6692-6760
					6805-7073
	1				7286-7514
			:		7744-7833
:				.	8003-8545
		ļ			8778-8913
					9249-9703
HSIGQ50	233	932448	AC019214	764	1-160
•					713-910
					1069-1269
					3997-4098
					4303-4397
					5035-5098
					5740-5796
					6024-6155
					6697-6813
					6937-7029
					7110-7349
					7432-7571
					7573-7601
_					7834-7907
		1			8326-8490
				•	8712-8804
					8894-8979
					9090-9171
	6	•			9368-9467
					9622-9730
					9821-10012
					10197-10277
			i		10440-10562
					10668-11103
					11203-11432
					11937-12052
					12251-12312
					12794-13183

					13257-13343
		· · · · · · · · · · · · · · · · · · ·			13483-13996
					14001-14146
					14369-14483
					14587-15046
					15053-15302
				•	15470-15534
		!			15624-15695
					16128-16212
					17904-17980
					18066-18189
		•			18298-18394
					18494-18574
					18668-18771
					18896-19043
					19245-19364
					19650-19925
					19968-20102
					20205-20354
					20529-21648
					21748-21816
					21861-22129
					22341-22569
					22799-22888
					23058-23600
					23833-23968
					24304-24757
HSIGQ50	233	932448	AC019214	765	1-803
					1028-1918
HNSMB24	235	971537	AC015555	766	1-61
					464-586
					752-1423
					3455-3587
			-		5766-5958
					6757-7115
					8075-8329
					8778-8876
					12309-12455
					13123-13279
					16212-17107
HNSMB24	235	971537	AP001623	767	1-61

					464-586
					752-1423
					3455-3580
					4976-5021
					5793-5958
					6757-7115
					8075-8329
					8778-8876
					12305-12451
					13119-13275
					16208-17104
HNSMB24	235	971537	AC015555	768	1-674
HNSMB24	235	971537	AP001623	769	1-674

Table 1B summarizes additional polynucleotides encompassed by the invention [43] (including cDNA clones related to the sequences (Clone ID NO:Z), contig sequences (contig identifier (Contig ID:) contig nucleotide sequence identifiers (SEQ ID NO:X)), and genomic sequences (SEQ ID NO:B). The first column provides a unique clone identifier, "Clone ID NO:Z", for a cDNA clone related to each contig sequence. The second column provides the sequence identifier, "SEQ ID NO:X", for each contig sequence. The third column provides a unique contig identifier, "Contig ID:" for each contig sequence. The fourth column, provides a BAC identifier "BAC ID NO:A" for the BAC clone referenced in the corresponding row of the table. The fifth column provides the nucleotide sequence identifier, "SEQ ID NO:B" for a fragment of the BAC clone identified in column four of the corresponding row of the table. The sixth column, "Exon From-To", provides the location (i.e., nucleotide position numbers) within the polynucleotide sequence of SEQ ID NO:B which delineate certain polynucleotides of the invention that are also exemplary members of polynucleotide sequences that encode polypeptides of the invention (e.g., polypeptides containing amino acid sequences encoded by the polynucleotide sequences delineated in column six, and fragments and variants thereof).

CABLE 2

Clone ID	Contig D:	SEQ ID	Analysis	PFam/NR Description	PFam/NR Accession Number	Score/ Percent	NT From	NT To
NO:Z			Method			Identity		
HFRBN59	1106393	1-1	blastx.14	Hypothetical fimbrial chaperone in pepN-pyrD intergenic region.	gi 4062512 dbj BAA3 5699.1	82%	212	445
HFRBN59	739539	237	HMMER 1.8	PFAM: Fimbrial proteins	PF00419	19.62	98	232
HE2KJ64	906019	12	HMMER 1.8	PFAM: SCP-like extracellular Proteins	PF00188	66.3	35	277
			blastx.14	(AF109674) late gestation lung protein 1 [Rattus norvegicus]	gi 4324682 gb AAD1 6986.1	74%	2	364
HAGDV32	1178626	13	blastx.2	Diacylglycerol kinase iota (Fragment).	sp AAF43006 AAF43 006	100%	19	243
HAGDV32	699372	238	HMMER 1.8	PFAM: Ank repeat	PF00023	15.79	41	121
HLICC37	856958	14	HMMER 2.1.1	PFAM: Ank repeat	PF00023	33.1	53	151
HBGBU96	1121900	15	blastx.2	hypothetical 30.8 kD protein in gltF-nanT intergenic region - Escherichia coli (strain K- 12)	pir H65113 H65113	%6L	3	449
HBGBU96	848220	239	HMMER 2.1.1	PFAM: ROK family	PF00480	65.2	3	125
НАЈСQ63	823850	16	HMMER 2.1.1	PFAM: Ank repeat	PF00023	6.96	175	267

					_	_																		
628	322	382	584	448	419	256	146	450	144	533	66	66	44	468	295	556	561	308	323	203	353	323	323	323
242	245	230	53/	404	378	173	66	151	1	447		19	3	7	197	131	499	216	186	57	195	174	219	192
63%	17.86	62%	81%	%98	71%	39%	43%	91%	100%	%68	30%	46%	13.97	72%	62.3	72%	47%	59.5	41%	34%	37%	36%	48%	43%
sp Q9UQR3 Q9UQR 3	PF00023	gi 488505 dbj BAA06	418.1					gi 5712756 gb AAD4	7636.1 AF160798_1				PF00023	sp CAB89816 CAB8 9816	PF00023	gi 5262748 emb CAB	45688.1	PF00023	gi 790608 gb AAA85	854.1				
CENTAURIN BETA2.	PFAM: Ank repeat	similar to HUMORFU	(D26069) [Homo sapiens]					(AF160798) calcium	transporter CaT1 [Rattus	norvegicus			PFAM: Ank repeat	Shank3b protein.	PFAM: Ank repeat	(AJ133120) Proline rich	synapse associated protein 2 [Rattus norvegicus]	PFAM: Ank repeat	UNC-44 [Caenorhabditis	elegans				
blastx.2	HMMER 1.8	blastx.14						blastx.14					HMMER 1.8	blastx.2	HMMER 2.1.1	blastx.14		HMMER 2.1.1	blastx.14					
17	240							18					241	19	242			20						
1153903	926188							1096389					959139	1152327	903653			924647						
HLMMV66	HLMMV66							HLWAR08					HLWAR08	HBGTT76	HBGTT76			HMCF024						

323 182 173 167 182 323 200 170 171 462	574	757	482	962	467	388	1915	1804	1585	478	762	1041	350	244
174 102 90 60 75 78 78 78 3 3	476	479	291	982	285	62	2088	2097	1728	531	1037	1085	06	191
34% 48% 38% 42% 47% 47% 50%	37.4	33%	31%	25%	29%	28%	53.39	%99	45%	%99	93%	%09	128.3	12.82
	PF00023	gb AAC96986.1				sp AAF67491 AAF67 491	PF00412	gi 841318 gb AAA85	718.1		gi 841318 gb AAA85	718.1[PF00684	PF00023
	PFAM: Ank repeat	contains 10 ankyrin-like	repeats; similar to human	ankyrin, 1 bursaria	Chlorella virus 1]	Sterol regulatory element binding protein 3.	PFAM: LIM domain containing proteins	mutant sterol regulatory	element binding protein-2		mutant sterol regulatory	element binding protein-2	PFAM: DnaJ central domain (4 repeats)	PFAM: Ank repeat
	HMMER 2.1.1	blastx.2				blastx.2	HMMER 1.8	blastx.14			blastx.14		HMMER 2.1.1	HMMER 1.8
	21					22	243				244		23	245
	973137					1012465	892926				975276		290990	823859
	HBIOM94		1	88		HBJLR11	HBJLR11				HBJLR11		HLTER04	HMSMU30

H2MBY83 752124	25	HMMER	PFAM: Protein of	PF01951	137	08	493
		2.1.1	unknown function				
		blastx.2	CG6353 PROTEIN.	sp Q9VD92 Q9VD92	58%	80	493
1164739	26	blastx.2	CDNA FLJ10852 FIS, CLONE NT2RP4001498,	sp BAA91856 BAA9 1856	51%	130	1299
	-		WEAKLY SIMILAR TO 1				
810424	246	HMMER 2.1.1	PFAM: Ank repeat	PF00023	35.4	340	438
975304	27	HMMER 2.1.1	PFAM: Putative GTP-ase activating protein for Arf	PF01412	196.5	362	739
		blastx.14	(AF124491) ARF	gi 4691728 gb AAD2	%68	368	1813
			GTPase-activating protein	8047.1 AF124491_1	100%	1964	2509
			. GIT2 [Homo sapiens]		%16	1712	1972
					25%	1730	1894
					34%	2000	2104
					33%	1280	1360
					29%	1445	1546
975378	28	HMMER 2 1 1	PFAM: LIM domain	PF00412	31.3	968	1009
		blastx 2	I.IM and cysteine-rich	sn A AF34411 A AF34	%06	116	1000
			domains protein 1.	411	34%	917	1021
971772	247	HMMER 2.1.1	PFAM: Ank repeat	PF00023	321.4	820	918
		blastx.2	ankyrin G [Homo sapiens]	gb AAA64834.1	%56	91	1239
					39%	163	1182
					37%	130	1182
					34%	163	1206
					36%	166	1185
					32%	106	1182
					33%	103	1188
					39%	163	609

148 597	1230 244	354 440	81 1031	34 75	1(148 519		25 1314																	
38%	100%	85	100%	100%	26%	147.9	%66		72.3	72.3	72.3	72.3 70% 48% 44%	72.3 70% 48% 44%	72.3 70% 48% 44% 44% 39%	72.3 70% 48% 44% 44% 39% 36%	72.3 70% 48% 44% 39% 36% 35%	72.3 70% 44% 44% 39% 36% 35%	72.3 48% 44% 39% 36% 36% 34% 57%	72.3 70% 48% 44% 39% 36% 35% 35% 57% 53%	72.3 70% 48% 44% 36% 36% 35% 34% 57% 53%	72.3 70% 48% 44% 39% 36% 35% 34% 57% 53% 53%	72.3 70% 48% 44% 36% 36% 36% 36% 36% 36% 36% 36	72.3 70% 48% 44% 39% 36% 35% 34% 57% 53% 53% 46%	72.3 48% 44% 36% 36% 36% 34% 57% 53% 53% 53% 53% 53% 53% 53%	72.3 70% 48% 44% 39% 36% 35% 35% 35% 32% 53% 53% 53% 53% 53% 53% 53% 53
	sp Q9Y576 Q9Y576	PF00023	gi 5306062 gb AAD4	1894.1 AF156777 1 .	}	PF01980	sp BAA91013 BAA9 1013		PF00023	PF00023 sp Q9VCA7 Q9VCA	PF00023 sp Q9VCA7 Q9VCA 7	PF00023 sp Q9VCA7 Q9VCA 7	PF00023 sp Q9VCA7 Q9VCA 7	PF00023 sp Q9VCA7 Q9VCA 7	PF00023 sp Q9VCA7 Q9VCA 7	PF00023 sp Q9VCA7 Q9VCA 7	PF00023 sp Q9VCA7 Q9VCA 7 PF01942	PF00023 sp Q9VCA7 Q9VCA 7 PF01942 gi 3128208 gb AAC2	PF00023 sp Q9VCA7 Q9VCA 7 PF01942 gi 3128208 gb AAC2 6688.1	PF00023 sp Q9VCA7 Q9VCA 7 PF01942 gi 3128208 gb AAC2 6688.1	PF00023 sp Q9VCA7 Q9VCA 7 PF01942 gi 3128208 gb AAC2 6688.1	PF00023 sp Q9VCA7 Q9VCA 7 PF01942 gi 3128208 gb AAC2 6688.1 PF02005			
	ASB-1 PROTEIN.	PFAM: Ank repeat	(AF156777) ASB-1	ens		PFAM: Uncharacterised I protein family	206 FIS, F1582.		PFAM: Ank repeat	•										+ 17	I. hetical				
	blastx.2 A	~	.14		4	HMMER PI 2.1.1		H		HMMER P. 2.1.1 blastx.2 C															
	30	248				31			32	32	32	32	35	32	32	35	32	32		33	33 33	33	33 33	33	33 33 33 34
	1152346	911597				919538			911586	911586	911586	911586	911586	911586	911586	911586	911586	911586	911586	911586	911586	911586	911586	911586	911586
	HNJCE31	HNJCE31				HKAIU14			HCE4112	HCE4112	HCE4112		HCE4112												

720	2310	2337	1043	1130	1249	237		207		327 339	391	1399	307	715	326
16	2104	529	336	405	1085	160		46		13 292	293	2	 38	320	78
35%	65.2	%66	191.2	%66	%08	100%		67.2		67%	50.4	62%	42.2	78%	%86
dbj BAA30948.1	PF00563	dbj BAA35528.1	PF01963	emb CAB63043.1				PF00412		gi 1710382 gb AAB3 8287.1	PF00023	sp 097902 097902	PF02000	pir A61382 A61382	-
(AP000007) 381aa long hypothetical N2,N2- dimethylguanosine tRNA methyltransferase [Pyrococcus horikoshii]	PFAM: Domain of unknown function 2	Hypothetical 67.7 kd protein CY02B10.18C. [Escherichia coli]	PFAM: TraB family	(AL022328) dJ402G11.4	(novel protein similar to	C. elegans F38A5.2	(isoform 1)) [Homo saniens]	PFAM: LIM domain	containing proteins	ajuba; jub [Mus musculus]	PFAM: Ank repeat	DIFFERENTIATION ENHANCING FACTOR	PFAM: Protein of	phosphorylation	regulatory protein HP-10 - human
blastx.2	HMMER 2.1.1	blastx.2	HMMER 2.1.1	blastx.2				HMMER	2.1.1	blastx.14	HMMER 2.1.1	blastx.2	HMMER 2.1.1	blastx.14	·
	35		36					249			38		39		
	945288		951601					946972			823854		972981		
	HFXKW18		HBIMF04				191	HEEAU28			HDPKI66		НОСОДО8		

HDPRP54	1228283	40	blastx.2	CDNA FLJ10852 FIS, CLONE NT2RP4001498, WEAKLY SIMILAR TO	sp BAA91856 BAA9 1856	%96	75	1517
HDPRP54	502892	250.	HMMER 1.8	PFAM: Ank repeat	PF00023	20.99	330	401
HE2BW32	609468	41	HMMER 2.1.1	PFAM: MSP (Major sperm protein) domain	PF00635	87.1	19	192
HAJAU21	909029	42	HMMER 2.1.1	PFAM: Adaptin N terminal region	PF01602	194.1	2	322
		•	blastx.2	gamma-adaptin precursor - mouse	pir A36680 A36680	%56	2	319
HE8DL23	693641	43	HMMER 2.1.1	PFAM: Adaptin N terminal region	PF01602	131.4	29	343
			blastx.2	GAMMA2-ADAPTIN.	sp 075504 075504	83%	29 408	406
HFTCM92	928851	44	HMMER 1.8	PFAM: LIM domain containing proteins	PF00412	43.25	178	351
			blastx.2	ALPHA-ACTININ-2 ASSOCIATED LIM PROTEIN.	sp 070209 070209	36%	22 22	357
HFTCM92	948605	251	blastx.14	carboxyl terminal LIM domain protein [Homo sapiens]	gi 1905874 gb AAC0 5580.1	64%	581	456
HE6BQ76	775616	45	HMMER 2.1.1	PFAM: Double-stranded RNA binding motif	PF00035	28.1	155	223
			blastx.2	PROTEIN ACTIVATOR OF THE INTERFERON- INDUCED PROTEIN KTNASF	sp 075569 075569	66% 94%	146	340 159
HAMFP60	715097	46	HMMER	PFAM: Clathrin adaptor	PF01217	150.3	164	460

	305	403	390	217	399	249	833	833	815	725	638	737	707	605	710	593	731	503	413	521	707	404	410	969	827
	129	846	283	2	211	190	735	126	126	123	123	339	1111	219	129	201	204	144	120	123	342	135	117	501	744
	78.2	36%	26.1	%88	%99	17.29	187.9	33%	32%	30%	31%	36%	30%	37%	29%	36%	25%	38%	36%	31%	32%	41%	29%	32%	42%
	PF01217	7V6V9Q17V9V9Q qs	PF00035	sp BAA91862 BAA9	1862	PF00023	PF00023	gi 557084 gb AAC37	208.1																
complex small chain	PFAM: Clathrin adaptor complex small chain	CG1800 PROTEIN.	PFAM: Double-stranded RNA binding motif	CDNA FLJ10860 FIS,	CLONE NT2RP4001568, WEAKLY SIMILAR TO	PFAM: Ank repeat	PFAM: Ank repeat	ankyrin [Drosophila	melanogaster]																
2.1.1	HMMER 2.1.1	blastx.2	HMIMER 2.1.1	blastx.2		HMMER 1.8	HMMER 2.1.1	blastx.14																	
	47	48	252	49		253	254																		
	715098	1150900	859840	1165338		944518	904598																		
	HHFHY84	HE6FD03	HE6FD03	HDTFT90		HDTFT90	HPJCU63		19																

815	833	737	836	824	026	812	349	352	136			246	1135			712	595	355	508	557	909	1458 278
726	738	009	732	732	923	726	176	68	14			148	2			443	2	257	2	525	514	562
40%	37%	32%	28%	35%	%95	34%	47.4	92%	85%			46.4	%66		_	102.6	61%	93.8	%66	% 06	28.1	%58 %96
							PF01454	sp 076058 076058	-		,	PF01602	sp BAA91511 BAA9	1511		PF00636	sp Q9Z1P7 Q9Z1P7	PF00023	gi 1136404 dbj BAA1	1489.1	PF01605	pir T34532 T34532
							PFAM: MAGE family	DJ1409.2	(MELANOMA-	ASSOCIATED	ANTIGEN MAGE LIKE).	PFAM: Adaptin N	CDNA FLJ10259 FIS,	CLONE	HEMBB1000947, HIGHLY SIMILAR TO 1	PFAM: RNase3 domain.	NG28.	PFAM: Ank repeat	similar to ankyrin of	Chromatium vinosum. [Homo sapiens]	PFAM: eRF1-like proteins	hypothetical protein DKFZp434B1517.1 -
							HMIMER	blastx.2				HMMER 211	blastx.2			HMMER 2.1.1	blastx.2	HMMER 2.1.1	blastx.14		HMMER 2.1.1	blastx.2
							51					52	53			255	54	256			55	56
							793203					605962	1158800			914398	1197903	932013			856922	1204696
							HFITE38					НДРДН64	F HFKKS58			HFKKS58	HE8CM38	HE8CM38			HAJBU67	HHEHD10

				human (fragment)				
нненр10	894411	257	HMMER 1.8	PFAM: LIM domain containing proteins	PF00412	28.72	48	224
HHEND45	919630	57	HMMER 1.8	PFAM: Double-stranded RNA binding motif	PF00035	12.86	25	114
HE8EQ22	1031960	58	blastx.2	ASB-3 PROTEIN (CDNA	sp Q9Y575 Q9Y575	82%	199	702
				FLJ10123 FIS, CLONE		94%	695	751
				HEMBA1002939, WEAKLY 1		42%	674	751
HE8EQ22	911594	258	HMMER 2.1.1	PFAM: Ank repeat	PF00023	128.9	430	528
			blastx.14	(AF156778) ASB-3	gi 5306064 gb AAD4	94%	199	982
				protein [Homo sapiens]	1895.1 AF156778_1	37%	433	615
						10%	792	884
						36%	718	783
HSACD83	911588	59	HMMER 2.1.1	PFAM: Ank repeat	PF00023	47	160	258
			blastx.2	WUGSC:H_DJ1035002.1	aneo emaneo ds	%85	169	402
				PROTEIN (FRAGMENT).	M3	53%	438	554
HHGB053	1091714	09	blastx.2	hypothetical protein	pir T34532 T34532	92%	235	402
				DKFZp434B1517.1 -		93%	685	771
				human (fragment)		21%	27	128
						25%	611	691
HHGBO53	894375	259	HMMER 1.8	PFAM: LIM domain containing proteins	PF00412	26.8	133	252
HE8FD82	1154785	61	blastx.2	Hypothetical 35.8 kDa protein.	sp CAC09448 CAC0 9448	%66	8	811
HE8FD82	909634	260	HMMER 2 1 1	PFAM: Putative GTP-ase	PF01412	184.7	256	618
			blastx.14	(AL031633) similar to	gi 3880859 emb CAA	57%	265	510

	_			Ank repeat (2 domains);	21032.1	42%	502	732
				cDNA 1	-	46%	733	861
						46%	128	172
HOHAS44	914810	62	HMMER 2.1.1	PFAM: 7-fold repeat in Clathrin and VPS	PF00637	104.3	2	379
		··	blastx.14	clathrin heavy chain [Bos taurus]	gi 969024 gb AAC48 524.1	100%	2	664
HE80F42	1117857	63	blastx.2	CDNA FLJ20636 FIS,	sp BAA91302 BAA9	28%	199	486
				CLONE KAT03434.	1302	36%	208	483
						35%	181	489
						21%	488	544
HE80F42	810432	261	HMMER 2.1.1	PFAM: Ank repeat	PF00023	47.6	298	396
HSKHS71	1154798	64	blastx.2	ankyrin repeat protein	pir T18184 T18184	40%	52	477
				A682L - Chlorella virus		33%	52	726
1,				PBCV-1		35%	4	504
96						32%	7	486
						31%	7	504
						34%	7	405
						27%	4	456
HSKHS71	911592	262	HMMER	PFAM: Ank repeat	PF00023	63.1	94	192
			blastx.14	contains 10 ankyrin-like	gi 2447128 gb AAC9	42%	106	366
				repeats; similar to human	6986.1	36%	97	357
	•	•		1 Paramecium bursaria		35%	103	372
				Chlorella virus 1]		35%	100	. 372
	· · · · · · · · · · · · · · · · · · ·					38%	103	357
						31%	103	366
						40%	196	381
						35%	1	84
HISBT75	1181020	65	blastx.2	LIM DOMAIN PROTEIN CLP-36.	sp 000151 CL36_HU MAN	40%	37	360

963281 263 H	HMMER 1.8	田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田田	PFAM: LIM domain containing proteins	PF00412	43.09	129	302
blastx.14	14		carboxyl terminal LIM domain protein [Homo sapiens]	gi 1905874 gb AAC0 5580.1	64% 25%	183	308
930964 66 HMMER 2.1.1	ER		PFAM: 7-fold repeat in Clathrin and VPS	PF00637	642.4	3205	2780
-	-	, –	clathrin heavy chain [Bos	gi 969024 gb AAC48	%86	3718	1748
			taurus]	524.1	100%	1741	1583
					36%	2014	1940
					39%	3788	3720
					46%	1936	1892
					48%	3535	3461
					21%	3289	3248
					30%	3196	3128
	_				46%	3558	3514
		Į			78%	2011	1937
931402 67 HMMER P 2.1.1 R		면 임	PFAM: Leucine Rich Repeat	PF00560	30.1	340	408
blastx.2			hypothetical protein	emb CAA73132.1	48%	115	456
\exists	\exists	_	Silene latifolia]		39%	115	456
932927 68 HMMER P		Д	PFAM: PWWP domain	PF00855	48.7	<i>L</i> 9	234
		_	WHSC1 PROTEIN.	sp O96028 O96028	62%	29	585
		I			929	546	605
933854 69 HMMER PI		\Box	PFAM: Repeat in	PF02134	110.2	487	654
		45	ubiquitin-activating (UBA) proteins				
blastx.14 S		\mathcal{O}	Sbx [Mus musculus]	gi 54058 emb CAA44	36%	. 4	285
				465.1	44%	421	654
					35%	277	336

935229 70	-				41%	642	713
					58%	707	742
		HMMER 2.1.1	PFAM: Leucine Rich Repeat	PF00560	69.4	757	825
	<u> </u>	blastx.14	similar to yeast adenylate cyclase (\$56776) [Homo sapiens]	gi 1504042 dbj BAA1 3220.1	51%		1176
894905 264		HMMER 1.8	PFAM: Laminin B (Domain IV)	PF00052	1.3	115	189
942490 72		HMMER 2.1.1	PFAM: Leucine Rich Repeat	PF00560	33.6	236	307
	1	blastx.14	proteoglycan I precursor	gi 306884 gb AAA36	%09	206	574
	<u> </u>		[Homo sapiens]	009.1	63%	111	224
					34%	120	206
					36%	84	158
					32%	114	206
870698 73		HMMER 1.8	PFAM: Double-stranded RNA binding motif	PF00035	36	208	669
	1	blastx.2	testis nuclear RNA	pir 148840 148840	87%	190	669
			binding protein - mouse	7	80%	716	1207
			•		%19	1179	1325
1128320 74		blastx.2	ankyrin-related protein	pir A57282 A57282	34%	71	475
			unc-44 - Caenorhabditis		31%	119	475
			elegans (fragment)		38%	197	469
823900 265		HMMER 2.1.1	PFAM: Ank repeat	PF00023	36.9	205	315
	<u> </u>	blastx.2	ankyrin 3 [Mus musculus]	gb AAB01605.1	37%	28	348
1213746 75		blastx.2	probable ATPase SKD3	pir 149045 149045	83%	256	1869
		ļ	[imported] - mouse		84%	112	393
947964 266		HMMER	PFAM: Ank repeat	PF00023	52	140	238

HNTSX71 1221117 76 blastx.2 hypothetical profein prif742678[742678 hypothetical profein human (fragment) human				blastx.14	(AB027570) suppressor of	gi 4958935 dbi BAA7	85%	8	289
HNTSX71 1221117 76 blastx.2 bypothetical proteins pirjT42678[742678 99% blastx.2 blastx.1 blastx.1 blastx.2 blastx.2 blastx.2 blastx.2 blastx.2 blastx.2 blastx.3 blastx.2 complex small chain and proteins blastx.3 blastx.2 complex small chain adaptor complex small chain adaptor complex small chain adaptor complex small chain blastx.2 complex small chain complex chain complex chain complex chain complex chain complex chain complex chain complex chain complex chain complex chain complex chain complex chain complex chain complex chain					potassium transport defect	8095.1	93%	380	472
HNTSX71 963289 267 HMMER PEAM: Linguistic Production Pro	HNTSX71	1221117	92	blastx.2	hypothetical protein DKFZp434G171.1 -	pir T42678 T42678	%66	824	1417
blastx.14 thyroid receptor interactor gil695374 gb AAC41 44% 974741 268 HMMER PFAM: LIM domain PF00412 54.6 2.1.1 containing proteins 740.1 47% 1.1.1 Repeat 740.1 44% 2.1.1 Repeat 740.1 44% 2.1.1 Repeat 740.1 44% 1.1.1 Repeat 740.1 44% 1.1.1 Repeat 740.1 44% 1.1.1 Repeat 740.1 740.1 47% 1.1.1 Repeat 740.1 740.1 740.1 1.1.1 Repeat 740.1 740.1 740.1 1.1.1 Repeat 740.1 740.1 75% 1.1.1 Repeat 740.1 71% 1.1.1 Repeat 740.1 74% 1.1.1 Repeat 740.1 74% 1.1.1 74% 16% 1.1.1 74% 16% 1.1.1 74% 16% 1.1.1 74% 1.1.1	HNTSX71	963289	267	HMMER 2.1.1	PFAM: LIM domain	PF00412	54.6	44	217
HNTSX71 974741 268 HMMER PFAM: LIM domain PF00412 54.6 HFCFH75 2.1.1 containing proteins gi[695374gb]AAC41 44% HFCFH75 951202 77 HMMER PFAM: Leucine Rich PF00560 88.3 HFCFH75 951202 77 HMMER PFAM: Leucine Rich PF00560 88.3 PFAM: Leucine Rich PFAM: Leucine Rich PF00560 88.3 DIGT/H15.1 (A novel sp[CAC04183]CAC0 88.3 PROPYSS 1204693 78 Inch 1 HEOQYSS 1204693 78 blastx.2 CG15118 PROTEIN. sp[Q9V8R1 Q9V8R1 49% HFDQYSS 883406 269 HMMER PFAM: Ank repeat PF00023 39.9 HFDQ48 952185 79 HMMER PFAM: Clathrin adaptor PF01217 163.3 HPJDQ48 952185 79 HMMER PFAM: Clathrin adaptor PF01217 163.3 L1.1 complex small chain blastx.2 (AB015320) sigma1B dbjBA33392.1				blastx.14	thyroid receptor interactor [Homo sapiens]	gi 695374 gb AAC41 740.1	44%	14 263	268
HFCFH75 951202 77 HMMER PFAM: Leucine Rich 740.1 44% 47% 1.1.1 Repeat 740.1 47% 47% 1.1.1 Repeat 740.1 47% 951202 77 HMMER PFAM: Leucine Rich PF00560 98.3 2.1.1 Repeat 9800CAC04183 CAC0 83% 33% 1.0.0 1 1.0.0 1 1.0.0 1 1.0.0 1 1.0.0 1 1.0.0 1 1.0.0 1 1.0.0 1 1.0.0 1 1.0.0 1 1.0.0 1.0.0 1 1.0.0 1.0.0 1 1.0.0 1.0.0 1 1.0	HNTSX71	974741	268	HMMER 2.1.1	PFAM: LIM domain containing proteins	PF00412	54.6	865	425
HFCFH75 951202 77 HMMER PFAM: Leucine Rich PF00560 98.3 1.11 Repeat 2.1.1 Repeat 32% protein similar to leucine 4183 33% rich 1 1204693 78 blastx.2 CG15118 PROTEIN. splQ9V8R1 Q9V8R1 49% HEOQY55 1204693 78 blastx.2 CG15118 PROTEIN. splQ9V8R1 Q9V8R1 49% HEOQY55 883406 269 HMMER PFAM: Ank repeat PF00023 39.9 HPJDQ48 952185 79 HMMER PFAM: Clathrin adaptor PF01217 163.3 AB015320) sigma1B dbjlBAA33392.1 74%				blastx.14	thyroid receptor interactor [Homo sapiens]	gi 695374 gb AAC41 740.1	44%	628	374 236
HEOQY55 1204693 78 HMMER PFAM: Clathrin adaptor PF01217	951202	77	HMMER 2.1.1	PFAM: Leucine Rich Repeat	PF00560	98.3	134	202	
Protein similar to leucine- 4183 32%	000			blastx.2	DJ677H15.1 (A novel	sp CAC04183 CAC0	83%	2	694
1204693 78 blastx.2 CG15118 PROTEIN. sp Q9V8R1 Q9V8R1 49% 183406 269 HMMER PFAM: Ank repeat PF0023 39.9 2.1.1					protein similar to leucine-	4183	32%	242	643
26% 25% 33% 33% 1204693 78 blastx.2 CG15118 PROTEIN. sp Q9V8R1 Q9V8R1 49% 43% 1204693 78 PFAM: Ank repeat PF00023 2.1.1 952185 79 HMMER PFAM: Clathrin adaptor PF01217 2.1.1 complex small chain blastx.2 (AB015320) sigma1B dbj BAA33392.1 74%					rich 1		33%	239	643
1204693							79%	5	628
1204693 78 blastx.2							25%	∞	643
1204693 78 blastx.2 CG15118 PROTEIN. sp Q9V8R1 Q9V8R1 49% 883406 269 HMMER PFAM: Ank repeat PF00023 39.9 952185 79 HMMER PFAM: Clathrin adaptor PF01217 163.3 2.1.1 complex small chain dbjBAA33392.1 74%							33%	410	712
1204693 78 blastx.2 CG15118 PROTEIN. sp Q9V8R1 Q9V8R1 49% 883406 269 HMMER PFAM: Ank repeat PF00023 39.9 952185 79 HMMER PFAM: Clathrin adaptor PF01217 163.3 2.1.1 complex small chain blastx.2 (AB015320) sigma1B dbj BAA33392.1 74%							83%	717	191
883406 269 HMMER PFAM: Ank repeat PF00023 39.9 952185 79 HMMER PFAM: Clathrin adaptor PF01217 163.3 2.1.1 complex small chain dbjBAA33392.1 74%	HEOQY55	1204693	78	blastx.2	CG15118 PROTEIN.	sp Q9V8R1 Q9V8R1	46%	175	1104
883406 269 HMMER PFAM: Ank repeat PF00023 39.9 952185 79 HMMER PFAM: Clathrin adaptor PF01217 163.3 2.1.1 complex small chain dbjBAA33392.1 74%							43%	1076	1690
883406 269 HMMER PFAM: Ank repeat PF00023 39.9 2.1.1 PFAM: Clathrin adaptor PF01217 163.3 2.1.1 complex small chain blastx.2 (AB015320) sigma1B dbj BAA33392.1 74%							30%	1031	1219
952185 79 HMMER PFAM: Clathrin adaptor PF01217 163.3 2.1.1 complex small chain dbjBAA33392.1 74%	НЕООҮ55	883406	569	HMMER 2.1.1	PFAM: Ank repeat	PF00023	39.9	244	342
(AB015320) sigma1B dbj BAA33392.1 74%	нРлDQ48	952185	62	HMMER 2.1.1	PFAM: Clathrin adaptor complex small chain	PF01217	163.3	254	541
				blastx.2	(AB015320) sigma1B	dbj BAA33392.1	74%	257	547

242	2041	1203	1969	646	646	162	134	455	467	1127	1016	1022	249	3	198
120	74	1394	74	29	2	64	63	9	60	1195	1771	1630 1642	317	854	842 851
73%	83%	90.3	%86	323	%96	57.5	55.1	21%	33%	32.7	93%	30%	89	92%	28%
	sp AAF59924 AAF59 924	PF00035	emb CAA59167.1	PF01602	gi 49878 emb CAA33 096.1	PF00023	PF00560	gb AAC28019.1		PF00560	sp CAC04183 CAC0	4183	PF00560	sp AAF77048 AAF77	048
subunit of AP-1 clathrin adaptor complex [Homo saniens]	Double-stranded RNA- binding protein p74.	PFAM: Double-stranded RNA binding motif	spermatid perinuclear RNA binding protein [Mus musculus]	PFAM: Adaptin N terminal region	A) (AA 1- culus]	PFAM: Ank repeat	PFAM: Leucine Rich Repeat	(AF062006) orphan G	protein-coupled receptor	PFAM: Leucine Rich Repeat	DJ677H15.1 (A novel	protein similar to leucinerich 1	PFAM: Leucine Rich Repeat	Erbb2-interacting protein	EKBIN,
	blastx.2	HMMER 2.1.1	blastx.2	HMMER 2.1.1	blastx.14	HMMER 2.1.1	HMMER 2.1.1	blastx.2		HMMER 2.1.1	blastx.2		HMMER 2.1.1	blastx.2	
	08	270		81		82	83			84			85		
	1174865	948595		953828		810429	955551			956045			956917		
	HTTCB17	HTTCB17		HE2SY09		HFEBN52	НСНМО62			HHSDM19			HDTIT49		

PCT/US01/01347

204	06	204	123	204	1034	100	428	529		535	959	1390		1366		2632				2956	387	098	1019	1618	1027	886			664
854	845	851	830	854	3	7,00	336	8		11	525	1148		143		2528				1898	4	819	954	1544	920	23			995
%96	26%	25%	25%	22%	100%	100	68.1	177.6		54%	47%	120.3		100%		263.8				%92	38%	64%	45%	36%	35%	%98			68.2
	-				sp BAA91250 BAA9	1230	PF00023	PF01902		sp Q9VYU1 Q9VYU	1	PF02020		gi 5106787 gb AAD3	9844.1	PF00806				ннуер/мниериниер	9	•				0.000000000000000000000000000000000000	9	•	PF00023
	-				CDNA FLJ20548 FIS,	CLOINE INTITIONS.	PFAM: Ank repeat	PFAM: Domain of	unknown function	CG1578 PROTEIN.		PFAM: eIF4-	gamma/eIF5/eIF2-epsilon	(AF083246) HSPC028	[Homo sapiens]	PFAM: Pumilio-family	RNA binding domains	(aka PUM-HD, Pumilio	homology domain)	PUM PROTEIN.						SCLE		PROTEIN (ANKYRIN REPEAT DOMAIN 1	PFAM: Ank repeat
					blastx.2	and of the	HMMER 211	HMMER	2.1.1	blastx.2		HMMER	2.1.1	blastx.14		HMMER	2.1.1			blastx.2						blastx.2			HMMER
					98	100	271	87				88				68										06			272
					1163072	707001	909887	958942				960253				963756						,				1189720			951627
					HTLGW19	TIME OXXIIO	HTLGW19	HJPCA88				HE9TA54				HCFCD40										HHBEN77			HHBEN77

			2.1.1			,		
			blastx.14	(AJ011118) skeletal	gi 5420272 emb CAB	84%	23	736
				muscle and cardiac	46646.1	34%	389	730
				protein [Mus musculus]		32%	404	629
						%08	736	798
HHESP66	1154641	91	blastx.2	CDNA FLJ20189 FIS, CLONE COLF0657.	sp BAA91003 BAA9 1003	94%	129	815
HHESP66	919192	273	HMMER 2.1.1	PFAM: Ank repeat	PF00023	53.2	428	526
НАННО37	967442	92	HMMER 2.1.1	PFAM: Leucine Rich Repeat	PF00560	63	494	562
			blastx.2	(AF053356) leucin rich	gb AAC78793.1	%96	104	1300
				neuronal protein [Homo		73%	1100	2011
				sapiens		100%	18	113
				1		42%	1186	1368
2/						36%	1270	1473
						28%	1129	1608
						78%	1626	2009
						36%	138	272
						36%	993	1115
						31%	376	989
						24%	1159	1419
HAMAA10	968749	93	HMMER 2.1.1	PFAM: Nebulin repeat	PF00880	105.2	628	726
			blastx.14	N-RAP [Mus musculus]	gi 2351568 gb AAC5	%98	613	915
					3323.1	52%	238	534
						%02	97	300
						41%	583	915
						40%	583	915
						48%	79	183
						48%	79	171
						28%	241	510

109	834	828	810	198	408	807	612	834	915	318	834	408	192	192	429	623	240	510	109	624	828	612	306	879	288	723	192	417	624	318	723
47	730	712	673	79	235	685	439	730	742	223	730	220	79	106	214	546	106	223	26	406	778	439	217	751	208	625	127	331	418	223	625
61%	42%	33%	32%	40%	31%	39%	22%	42%	34%	46%	37%	25%	31%	41%	22%	46%	33%	25%	61%	20%	28%	22%	36%	32%	37%	30%	54%	27%	. 23%	31%	30%
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			-		 					-			• • • • • • •			-,-	··	_ ;										· · ·		~	

915	915	834	309	192	189	585	318	840	393	171	318	573	723	318	189	504	294	426	915	732	915	828	810	828	396	. 177	201	405	402	915	177
838	838	778	220	121	121	439	220	90/	223	121	223	532	643	217	121	439	223	280	874	685	844	784	269	685	244	124	142	337	340	856	73
42%	38%	42%	36%	41%	47%	79%	27%	31%	22%	52%	25%	21%	37%	73%	39%	45%	33%	20%	64%	43%	33%	53%	31%	27%	19%	38%	30%	30%	38%	40%	25%
		······································	·	****							•											•••••									
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							<u></u>									204															

1882	2101	2162	1349		1097	1625	1628	1019	1454	1199	1163	1385	1313	1241	1166	1460	1379	1001	1232	1637	1454	1226	1379	1313	947	644	938
1814	167	2100	1278		543	1095	1062	543	009	786	714	918	846	786	792	1002	1002	702	858	1194	1074	918	1017	1041	648	552	282
68.8	28%	71%	132.1		35%	33%	32%	33%	31%	37%	31%	79%	30%	30%	34%	30%	31%	32%	32%	29%	767	34%	27%	28%	32%	28.9	%66
PF00560	dbj BAA91631.1		PF00560		gb AAD25540.1 AF1	33730_1																				PF01454	gb AAD31314.3 AF1 43235_1
PFAM: Leucine Rich Repeat	(AK001332) unnamed	protein product [Homo sapiens]	PFAM: Leucine Rich	Repeat	(AF133730) Slit1 [Rattus	norvegicus]																				PFAM: MAGE family	(AF143235) apoptosis related protein APR-1
HMMER 2.1.1	blastx.2		HMMER	2.1.1	blastx.2																					HIMMER 2.1.1	blastx.2
94			95													<u>-</u>										96	
969324			971339														-									613679	
HHFMH12			HDTIE58				_							05												HIBCN93	

	499	254	260	131	504		498	627	1694	1059	1194	173	1086	79	1032	42	1029	254	266	1.70		
	678	81	135	69	119		623	229	609	196	151	6	946	32	970		952	6	3	0		
	55%	43.69	64%	38%	43.78		64%	35%	46%	37.8	83%	75%	39%	87%	52%	64%	34%	86.4	95%			
	sp 000151 CL36_HU MAN	PF00412	gi 1905874 gb AAC0	5580.1	PF00412		gi 1905874 gb AAC0	5580.1	sp Q9Z1P7 Q9Z1P7	PF00023	oi11136404ldhilBAA1	1489.1						PF00790	sp Q9UJY5 Q9UJY5			
Homo sapiens	LIM DOMAIN PROTEIN CLP-36.	PFAM: LIM domain containing proteins	carboxyl terminal LIM	domain protein [Homo	PFAM: LIM domain	containing proteins	carboxyl terminal LIM	domain protein [Homo sapiens]	NG28.	PFAM: Ank repeat	similar to ankorin of	Chromatium vinosum.	[Homo sapiens]	1				PFAM: VHS domain	ADP-RIBOSYLATION	PROTEIN GGA1	(GAMMA-ADAPTIN	RELATED PROTEIN,
	blastx.2	HMMER 1.8	blastx.14		HMMER	1.8	blastx.14		blastx.2	HMMER	hlastx 14							HMMER 2.1.1	blastx.2			
	76	274			275				86	276								66				
	1165386	947000			948606				1216549	958555					,			812164				
	HSWAP86	HSWAP86			HSWAP86				HHSGI32	ł	206							HAJBH69				

266 87	797 84	223 462	69 269	10 1010 1039	617 715	566 766 796 864	729 430	774 415	4 180	4 735 82 711	89 247
46.8	%66	9.69	85.6	100%	33.7	49%	43.9	100%	95.6	97% 30%	40.2
				·							
PF01354	sp BAA91818 BAA9 1818	PF01041	PF01761	sp Q9UHR3 Q9UHR 3	PF00023	gi 1710175 gb AAB3 8316.1	PF01852	sp Q9Y5P4 Q9Y5P4	PF00412	sp CAB86657 CAB8 6657	PF01016
PFAM: Antifreeze protein	CDNA FLJ10797 FIS, CLONE NT2RP4000657, WEAKLY SIMILAR TO 1	PFAM: DegT/DnrJ/EryC1/StrS family	PFAM: 3-dehydroquinate synthase	NASOPHARYNGEAL CARCINOMA SUSCEPTIBILITY PROTEIN LZ16.	PFAM: Ank repeat	BRCA1-associated RING domain protein [Homo sapiens]	PFAM: START domain	GOODPASTURE ANTIGEN-BINDING PROTEIN (EC 2.7.1.37).	PFAM: LIM domain containing proteins	DJ393D12.2 (novel LIM domain protein).	PFAM: Ribosomal L27
HMMER 2.1.1	blastx.2	HMMER 2.1.1	HMMER 2.1.1	blastx.2	HMMER 2.1.1	blastx.14	HMMER 2.1.1	blastx.2	HMMER 2.1.1	blastx.2	HIMMER 2.1.1
100		101	102	103	277		104		105		106
923606		575037	702735	1179713	961297		855538		870561	_	872262
HAGFN07		HFRBZ64	HMAER78	HKAAV49	HKAAV49		HAPQS74		HTEPM33		HLTES49

HDTEJ81	919873	107	HMMER	PFAM: Ribosomal L27	PF01016	58.8	106	333
			2.1.1	protein				
			blastx.2	HSPC250.	sp AAF36170 AAF36 170	85%	16	474
HTLCY21	910212	108	HMMER 2.1.1	PFAM: LIM domain containing proteins	PF00412	132.9	230	412
			blastx.2	LIM/HOMEOBOX	sp P53776 LHX4 M	100%	101	538
		<u> </u>		PROTEIN LHX4.	OÙSE	%9L	478	750
		·				33%	227	397
						34%	/1/	208
HKAKF45	1090988	109	blastx.2	hypothetical protein	pir T17278 T17278	%88	-	831
				DKFZp434E1335.1 -		40%	28	465
				human (fragment)		30%	009	839
						35%	244	456
HKAKF45	911611	278	HMMER 2.1.1	PFAM: Ank repeat	PF00023	94.9	295	393
08	***		blastx.14	(AJ011118) skeletal	gi 5420272 emb CAB	36%	7	414
				muscle and cardiac	46646.1	28%	106	459
				protein [Mus 1				
HMWDF88	692906	110	HMMER	PFAM: Low-density	PF00057	41.61	171	245
			1.8	lipoprotein receptor domain class A				
			blastx.2	8D6 antigen.	sp AAF61850 AAF61 850	82%	6	242
HHECU86	945062	111	HMMER 2 1 1	PFAM: Glucose inhibited division profession A	PF01134	261.8	199	528
			blastx 2	HYPOTHETICAL.	splO9V2Z2IVC02 H	%16	313	528
				PROTEIN CGI-02.	UMAN	83%	527	616
HTPH001	1152424	112	blastx.2	LIM and cysteine-rich	sp AAF34411 AAF34	%98	11	646
				domains protein 1.	411			
HTPHO01	912348	279	HMMER	PFAM: LIM domain	PF00412	79.1	466	639

			2.1.1	containing proteins				
			blastx.14	testin [Mus musculus]	gi 475210 emb CAA5	48%	241	621
					5590.1	63%	4	141
						31%	337	498
						29%	541	612
						32%	358	432
HFXKR90	948399	113	HMMER 2.1.1	PFAM: TB domain	PF00683	31.6	240	347
			blastx.2	hypothetical protein	pir[T17298 T17298	83%	168	482
				DKFZp586M2123.1 -		%16		135
				human (fragment)		40%		132
						35%	180	365
						44%		132
						26%	43	132
		····-				33%	-	132
						52%	82	132
09						20%	138	191
						%69	532	570
	****					25%		84
нрРВQ32	949191	114	HMMER 2.1.1	PFAM: START domain	PF01852	58.9	396	719
			blastx.2	GTT1.	sp AAF81750 AAF81 750	94%	129	1004
HNTAR73	949289	115	HMMER 2.1.1	PFAM: TB domain	PF00683	28.7	131	235
			blastx.2	Latent transforming	sp AAF62352 AAF62	82%	2	286
				growth factor beta binding	352	47%	173	367
				protein 3.		61%	6	101
						17%	283	309
				· · · · · · · · · · · · · · · · · · ·		71%	5	25
HHEGC16	950778	116	HIMIMER 2 1 1	PFAM: Glucose inhibited division protein A	PF01134	263.1	1136	429
_		_	7:1:7					

			blastx.2	HYPOTHETICAL	sp Q9Y2Z2 YC02_H	%66	1136	348
				PROTEIN CGI-02.	UMAN	%16	1495	1139
HSIGE72	952275	117	HIMMER	PFAM: Molybdenum	PF00994	720.4	362	1645
			2.1.1	cofactor biosynthesis protein				
			blastx.2	gephyrin - rat	pir JH0681 JH0681	99%	182	1663
HCGMG56	953660	118	HMMER 2.1.1	PFAM: Ribosomal L27 protein	PF01016	47	119	241
			blastx.2	HSPC250.	sp AAF36170 AAF36 170	84%	29	487
HNGBQ66	966001	119	HMMER 2.1.1	PFAM: Putative snoRNA binding domain	PF01798	327.1	1020	1466
			blastx.2	NUCLEOLAR PROTEIN NOP5/NOP58	sp Q9Y2X3 Q9Y2X3	93%	264	1529
210		,		(NUCLEOLAR PROTEIN 5).				
HTXPY09	966013	120	HMMER 2.1.1	PFAM: Putative snoRNA binding domain	PF01798	39.1	465	551
			blastx.2	hypothetical protein	pir T17299 T17299	72%	327	587
				DKFZp564H2171.1 - human (fragment)		%99	103	237
HCHAS12	966626	121	HMMER 2.1.1	PFAM: START domain	PF01852	58.6	562	1014
			blastx.2	CGI-52 PROTEIN.	sp Q9Y365 Q9Y365	%69 %06	262	1209
H6EDI12	1154053	122	blastx.2	CG5891 PROTEIN.	XUV9QIVVV9QIqs	40%	165	533
H6EDI12	911587	280	HMMER	PFAM: Ank repeat	PF00023	73.3	274	372
			2.1.1 blastx.14	130 kDa myosin-binding	gi 633040 dbj BAA07	51%	277	516

507 237	493	961	239	236	236	236	236	239	239	239	254	239	239	251	236	224	290	790	284	296	254	332	.236	230	290	239	254	260
274	260	236	3	15	36	9	99	21	63	6	72	24	21	9	9	84	102	6	102	42	159	75	93	39	12	21	12	30
38%	39.8	61%	72%	28%	33%	76%	31%	79%	33%	23%	79%	762	22%	22%	26%	23%	30%	20%	23%	24%	21%	23%	767	22%	70%	70%	70%	19%
202.1	PF00435	sp Q9UPN3 Q9UPN3																										
subunit of smooth muscle myosin phophatase (M130) [Gallus gallus]	PFAM: Spectrin repeat	ACTIN BINDING	PROTEIN ABP620.											-														
	HMMER 2.1.1	blastx.2																										
	123																											
	911474					,																						
	HE8MI76												211															

3 2189		3 476		551				1	3 568	179	577								1742	989	3 932	5 496			t 825		4 632
3		3		12				982	323	12	332			165	2985				1641	564	888	98			184		234
%66		182.3		%68				%56	%16	71%	73.51			21%	%09	54%	35%	31%	38%	75%	%09	206.25			85%		245.38
gi 434759 dbj BAA04	699.1	PF00822		sp 060359 CCG3_H	UMAN			sp P49411 EFTU_HU	MAN		PF00009			pir T41396 T41396								PF00009			pir S40780 S40780		PF00009
similar to human	elongation factor 2 mRNA (HSEF2). [Homo sapiens]	PFAM: PMP-	22/EMP/MP20/Claudin family	VOLTAGE-	DEPENDENT	CALCIUM CHANNEL	GAMMA-3 SUBUNIT 1	ELONGATION FACTOR	TU, MITOCHONDRIAL	PRECURSOR (P43).	PFAM: Elongation factor	Tu family (contains	ATP/GTP binding P-loop)	probable translation	elongation factor EF-Tu -	fission yeast	(Schizosaccharomyces	pombe)				PFAM: Elongation factor	Tu family (contains	AIP/GIP binding P-loop)	translation elongation factor EF-G.	mitochondrial - rat	PFAM: Elongation factor
blastx.14		HMMER	2.1.1	blastx.2				blastx.2			HMMER	1.8		blastx.2								HMMER	1.8		blastx.2		HMMER
	·· - ·	128						129			286			130								287			131		288
		951351						1202534			840952			1226739								574258			1199942		882335
		HAGBX32						HLWEE80			HLWEE80		2	E HMEF181							.,	HMEF181			HOUHW83		HOUHW83

				ATP/GTP binding P-loop)				
HSLCB60	1193050	132	blastx.2	ribosomal protein S7 [validated] - Escherichia coli	pir H65127 R3EC7K	100%	820	284
HSLCB60	730740	289	HMMER 2.1.1	PFAM: Elongation factor Tu family	PF00009	197	115	450
HSLFG64	1228145	133	blastx.2	sulfate adenylyltransferase (EC 2.7.7.4) large chain - Escherichia coli	pir JN0327 JN0327	92%	856	2142
HSLFG64	853387	290	HMMER 2.1.1	PFAM: Elongation factor Tu family	PF00009	347.8	1127	54
HTPFX16	974296	134	HMMER 2.1.1	PFAM: PMP- 22/EMP/MP20/Claudin family	PF00822	50.2	48	299
			blastx.2	CLAUDIN-18.	sp P56857 CLDI_MO USE	67%	39	359 483
HWAER24	934693	135	HMMER 2.1.1	PFAM: Elongation factor G C-terminus	PF00679	88.1	2156	2398
			blastx.2	probable translation elongation factor EF-Tu -	pir T41396 T41396	60%	2045	2554
, (1 <u>1</u>) -				fission yeast		31%	1412	1918
				(Schizosaccharomyces		35%	 (C)	248
				pombe)		33%	707	834
HKMAC08	1121865	136	blastx.2	CAPACITATIVE CALCIUM ENTRY CHANNEL 1	sp P79100 P79100	%98	193	720
·,·				(CAPACITATIVE CALCIUM 1				
HKMAC08	888096	291	HMMER 2.1.1	PFAM: Ank repeat	PF00023	25.5	400	474

720	150	143	362	365	436	2	355	889		459		1685	. '7/	292	186	151
193	1	9	3	3	404	322	450	326		388		432		7	91	564
86%	· %86	41.58	237.9	%86	72%	%86	100%	135.4		30.2		94%		99.1	40.3	100%
gi 1731930 emb CAA 68125.1	pir B65104 B65104	PF00419	PF00267	gi 1651450 dbj BAA3	5675.1	pir E65006 E65006		PF00345		PF00057		pir F64892 F64892		PF00480	PF00480	pir F64851 F64851
capacitative calcium entry channel 1 [Bos taurus]	hypothetical fimbrial-like protein in agai-mtr intergenic reg - Escherichia coli (strain K-	PFAM: Fimbrial proteins	PFAM: General diffusion Gram-negative porins	Outer membrane protein f	precursor (outer membrane 1	hypothetical protein	b2335 - Escherichia coli (strain K-12)	PFAM: Gram-negative	pili assembly chaperone	PFAM: Low-density	inpoprotein receptor domain class A	probable membrane	protein b1411 - Escherichia coli	PFAM: ROK family	PFAM: ROK family	flagellar protein flgJ - Escherichia coli
blastx.14	blastx.2	HMMER 1.8	HMMER 2.1.1	blastx.14		blastx.2	!	HMMER	1.8	HMMER	7.1.1	blastx.2		HMMER 2.1.1	HMMER 2.1.1	blastx.2
	137	292	138			139		293		140		141		294	295	143
	1105323	791608	963457			882817		883338		949568		1107898		860207	531702	1151503
	HSLHS93	HSLHS93	HBGOT10			HSDJW73	215	HSDJW73		HWMEQ37		HFRBX44		HFRBX44	HRDDR74	HPIAQ70

へかいます。	97/3604	596	HMMER	PFAM: Flagella basal	PF00460	41.51	206	298
			1.8	body rod proteins				
			blastx.14	Flagellar hook-associated	gi 1651528 dbj BAA3	77%	322	498
				protein 1 (hap1). Escherichia coli1	5891.1	100%	194	322
HROAZ07 9	973603	144	HIMMER	PFAM: Flagella basal	PF00460	33.9	5	16
			1.8	body rod proteins				
			blastx.2	Molybdopterin-converting factor 16k chain	dbj BAA35443.1	%02	113	469
HTTER50 1	1220586	145	blastx.2	Sec61 alpha isoform 2.	sp AAF66696 AAF66	%66	64	1206
				•	969	%99	1202	1273
HTTER50 7	724581	297	HMMER	PFAM: eubacterial secY	PF00344	28.69	356	559
			1.8	protein				
HUFBV44	1220585	146	blastx.2	Sec61 alpha isoform 2.	sp AAF66696 AAF66 696	100%	15	338
HUFBV44	851306	298	HMMER	PFAM: eubacterial secY	PF00344	25.6	22	288
			1.8	protein				
HE2EI69 5	534587	147	HMMER	PFAM: Gram-negative	PF00345	41.12	152	331
\dagger			1.0	pin assembly chaperone				
HWMJR63 1	1152429	148	blastx.2	LIM and cysteine-rich	sp AAF34411 AAF34	 %06	117	1004
	,			domains protein 1.	411	34%	918	1004
HWMJR63 9	922134	667	HMMER	PFAM: LIM domain	PF00412	31.3	893	1006
			2.1.1	containing proteins				
			blastx.14	testin [Mus musculus]	gi 475210 emb CAA5	62%	377	709
					5590.1	21%	608	1006
						48%	221	370
						34%	905	1000
						32%	926	1000
HSLFD83 (6	667155	149	HMMER 2.1.1	PFAM: SUA5/yciO/yrdC family	PF01300	135.1	18	305

322 495	214 20	5 313	761 318	3 404		24 149				
53.39	%08	168.3	%86	70.6		110.7	110.7 72% 61% 72%	110.7 72% 61% 72% 42.4	110.7 72% 61% 72% 42.4	72% 61% 72% 42.4 42.4 99%
PF00412	gi 841318 gb AAA85 718.1	PF02005	pir A25336 WQEC2 G	PF00563	F 0000 TH	PF02005	PF02005 sp 076103 076103	PF02005 sp O76103 O76103 PF00652	PF02005 sp O76103 O76103 PF00652 sp Q9UIV5 Q9UIV5	PF02005 sp O76103 O76103 PF00652 sp Q9UIV5 Q9UIV5 PF02005
PFAM: LIM domain containing proteins	mutant sterol regulatory element binding protein-2	PFAM: N2,N2-dimethylguanosine tRNAmethyltransferase	e (EC	PFAM: Domain of unknown function 2		PFAM: N2,N2-dimethylguanosine tRNA methyltransferase	PFAM: N2,N2-dimethylguanosine tRNA methyltransferase R29425_1.	dimethylguanosine tRNA methyltransferase R29425_1. PFAM: Similarity to lectin domain of ricin beta-chain, 3 copies.	dimethylguanosine tRNA methyltransferase R29425_1. PFAM: Similarity to lectin domain of ricin beta-chain, 3 copies. UDP- GALNAC:POLYPEPTID E N- ACETYLGALACTOSA MINYLTRANSFERASE.	dimethylguanosine tRNA methyltransferase R29425_1. PFAM: Similarity to lectin domain of ricin beta-chain, 3 copies. UDP- GALNAC:POLYPEPTID E N- ACETYLGALACTOSA MINYLTRANSFERASE. PFAM: N2,N2- dimethylguanosine tRNA methyltransferase
 	blastx.14	HMMER 2.1.1	blastx.2		H	HMMER 2.1.1				
300	301	151	152	302		153	153	153	153	153
952737	956567	754641	756908	827518	0000	8//11/	8//11/	881286	881286	881286
НВКDА90	HBKDA90	HTLAA37	HTRAA36	HTRAA36	HRGDD16		217			

HMSII43	946985	156	HMMER	PFAM: SCP-like	PF00188	91.46	98	478
			1.8	extracellular Proteins				
			blastx.2	CG2337 PROTEIN.	sp Q9VI35 Q9VI35	51%	95	517
HMADV11	920770	157	HMMER 2.1.1	PFAM: SUA5/yciO/yrdC family	PF01300	191.3	36	422
			blastx.2	probable translation factor	pir F64874 F64874	%26	27	416
				yciO - Escherichia coli		100%	5	25
HNTCK35	1226201	158	blastx.2	SEX-DETERMINATION	sp Q9Z2G1 Q9Z2G1	%6 <i>L</i>	109	1449
	!			PROTEIN HOMOLOG FEM1A.		85%	1460	2113
HNTCK35	266599	303	HMMER 1.8	PFAM: Ank repeat	PF00023	26.8	229	297
			blastx.14	(AF064447) sex-	gi 3930525 gb AAC8	%19	109	309
				determination protein	2372.1	94%	309	359
				homolog Fem1a [Mus				•
218	100001	7		Illusculus I A TEL OFICEA TIONI) TIOPOOL) TIOPOOL	/022		000
HIFUQIO	102//81	601	Dlastx.2	LAIE GESTATION LUNG PROTEIN 1.	spiczencologo	0///	 00	780
HTPGQ16	909618	304	HMMER	PFAM: SCP-like	PF00188	19.96	148	603
			1.8	extracellular Proteins				
HOCMS18	1227594	160	blastx.2	hypothetical protein DKFZp434N161.1 -	pir T17268 T17268	100%	2433	2729
				human		-		
HOCMS18	961424	305	HMMER 2.1.1	PFAM: Ank repeat	PF00023	78.9	1102	1200
			blastx.14	similar to ankyrin of	gi 1136404 dbj BAA1	54%	649	1401
				Chromatium vinosum.	1489.1	42%	325	474
				[Homo sapiens]		35%	415	498
						79%	589	657
						37%	418	489
HE8AM58	1204936	161	blastx.2	LIM/HOMEOBOX	sp 035652 LHX8 M	84%	10	921

928	271	299	432	104	101	332	399	30	36	69	51	129	36	771	878	1041	1269	3107	414	741	792	777	738	777	186
875	86	27	76	223	397	397	200	1577	1676	2093	1874	2114	1775	2096	2117	1961	2117	3166	316	148	163	169	148	169	163
83%	89.5	43.7	91%	43.1	51%	81%	55%	20%	44%	32%	31%	78%	31%	28%	28%	30%	30%	25%	176.9	37%	33%	33%	34%	33%	33%
OUSE	PF00412	PF02000	pir A61382 A61382	PF02094	pir S49326 S49326			pir T42691 T42691											PF00023	gi 710551 gb AAB01	605.1				
PROTEIN LHX8 (L3).	PFAM: LIM domain containing proteins	PFAM: Protein of unknown function	phosphorylation regulatory protein HP-10 - human	PFAM: TS-N domain	nascent polypeptide-	associated complex alpha	chain - human	hypothetical protein	DKFZp434D2328.1 -	human (fragment)									PFAM: Ank repeat	ankyrin 3 [Mus musculus]					
	HMMER 2.1.1	HMMER 2.1.1	blastx.2	HMMER 2.1.1	blastx.2			blastx.2			-		-						HMIMER 2.1.1	blastx.14			_		
	306	162		163				164											307						
	894346	955542		998096				1226251											911616						
	HE8AM58	HUSGZ51		HELEQ48				HOFOE03		21									HOFOE03						

																								_							
777	141	111	738	681	747	732	675	753	777	777	582	276	486	143	143	137	143	086	086	137	086	143	959	378	143	086	956	143	143	959	143
169	121	140	163	295	169	160	178	277	232	340	181	163	163	m	m	m	n	855	852	27	849	27	852	148	27	928	928	27	15	849	27
32%	52%	5170	33%	41%	31%	78%	31%	33%	31%	35%	32%	31%	33%	40%	38%	44%	36%	33%	37%	48%	36%	46%	44%	32%	41%	37%	51%	38%	37%	35%	41%
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143	143	953	143	086	086	947	831	831	143	947	143	226	134	086	086	947	086	831	831	086	143	831	831	831	831	831	831	329	808	792
27	27	876	27	870	849	928	739	739	27	852	c	915	27	873	876	852	870	733	692	870	27	992	992	992	269	775	772	180	122	739
41%	41%	46%	41%	32%	25%	20%	45%	38%	35%	31%	27%	52%	33%	25%	31%	31%	762	33%	52%	29%	30%	40%	36%	36%	31%	42%	45%	67.1	91%	72%
														,						-								PF02119	sp AAF44722 AAF44	722
																												PFAM: Flagellar P-ring protein	Novel retinal pigment	epithelial cell protein.
												-																HMMER 2.1.1	blastx.2	
																												165	166	
															·													585289	1205511	
																221												HNFFR23	HOGCC57	

572 670	164 772		739 792	109 282		97 288	330 428	_	6 527															
116.7	20%	78%	38%	43.54		54%	114.1		63%	63%	63% 33% 36%	63% 33% 36% 39%	63% 33% 36% 39% 34%	63% 33% 36% 34% 34%	63% 33% 36% 39% 34% 32%	63% 33% 36% 39% 34% 32% 33%	63% 33% 36% 34% 32% 33% 33%	63% 33% 36% 34% 32% 33% 35%	63% 33% 36% 34% 34% 32% 33% 31% 31%	63% 33% 36% 34% 34% 32% 33% 31% 30%	63% 33% 36% 39% 34% 32% 33% 35% 31% 30% 80.4	63% 33% 36% 39% 34% 32% 33% 33% 31% 30% 80.4 80.4	63% 33% 36% 39% 34% 34% 32% 33% 31% 30% 80.4 80.4 80.4	63% 33% 36% 39% 34% 34% 32% 33% 35% 31% 30% 80.4 80.4 80.4 80.4 80.4 80.4 80.4 80.4
PF00023	gi 1429314 emb CAA	67582.1		PF00412		sp 000151 CL36_HU MAN	PF00023		pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691	pir T42691 T42691 PF01556 sp O75953 O75953	pir T42691 T42691 PF01556 sp O75953 O75953 PF00560	pir T42691 T42691 PF01556 sp O75953 O75953 PF00560 sp O75427 O75427	pir T42691 T42691 PF01556 sp O75953 O75953 PF00560 sp O75427 O75427
PFAM: Ank repeat	1 thyroid		stimulation [Canis familiaris]	п		LIM DOMAIN PROTEIN CLP-36.	PFAM: Ank repeat		hypothetical protein		ī	ı	ı	ı	ı	1	1	ı	1	etical protein 9434D2328.1 - (fragment) : DnaJ C terminal		etical protein 9434D2328.1 - (fragment) DnaJ C terminal SHOCK SIN HSP40-3.	etical protein 9434D2328.1 - (fragment) : DnaJ C terminal SHOCK =IN HSP40-3. : Leucine Rich	etical protein 9434D2328.1 - (fragment) : DnaJ C terminal SHOCK EIN HSP40-3. : Leucine Rich NRICH ONAL PROTEIN.
HMMER P	14		# S 4	HMMER P	\dashv	blastx.2 L	HMMER P		2	2	2	2.	.2	7	7.	7.	7	7			.2 .2 .2			
308				167			168													169	169	169	169	169
911609	_			926685	٠		823872													708477	708477	708477	708477	708477
HOGCC57				HFOZC96			HOHBK44	_					222							HHERB37				

723	301	476	930	479	482	476	930	930	476	470	470	476	470	467	443	876	006	927	470	431	180	876	482	744	927	470	332	473	780
151	203	3	475	9	9	9	490	511	24	9	15	co.	9	9	105	490	481	478	3	9	478	511	9	478	472	9	111	294	472
87%	32	999	20%	36%	34%	35%	34%	36%	32%	34%	31%	73%	29%	36%	35%	36%	33%	32%	78%	29%	29%	31%	28%	32%	29%	28%	37%	38%	27%
sp BAA95081 BAA9 5081	PF00023	pir T42691 T42691																											
Hypothetical 96.1 kDa protein.	PFAM: Ank repeat	hypothetical protein	DŘFZp434D2328.1 -	human (fragment)																		٠							
blastx.2	HMMER 2.1.1	blastx.2				· · · · · · · · · · · · · · · · · · ·																							
171	309	172																											1
1152264	823871	1152272																											
HDTDQ51	нртрQ51	HOHCG42						•																					

636	382	436	292	457	321	456	220	195	351	276	537	396	537	420	402	435	429
478	305	371	08	353	166	151	2	26	73	205	. 52	52	52	22	70	43	37
35%	11.27	59.8	97.6	08	36.45	%08	46%	31.7	48%	2.99	36%	39%	35%	41%	38%	37%	32%
	PF00023	PF00560	PF01454	PF00806	PF00412	pir T37192 T37192	717799V777	PF00023	dbj BAA11489.1	PF00560	pir JC5239 JC5239						
	PFAM: Ank repeat	PFAM: Leucine Rich Repeat	PFAM: MAGE family	PFAM: Pumilio-family RNA binding domains (aka PUM-HD, Pumilio homology domain)	PFAM: LIM domain containing proteins	nebulin-related protein, skeletal muscle - mouse	BCDNA:GH03482 PROTEIN.	PFAM: Ank repeat	similar to ankyrin of Chromatium vinosum. [Homo sapiens]	PFAM: Leucine Rich Repeat	insulin-like growth factor	acid-labile chain - baboon					
	HMMER 1.8	HMMER 2.1.1	HMMER 2.1.1	HMMER 2.1.1	HMMER 1.8	blastx.2	blastx.2	HMIMER 2.1.1	blastx.2	HMMER 2.1.1	blastx.2						
	310	173	174	175	176		117	311		178							
	887839	718918	731732	742053	969106		1152422	950367		827573							
	HOHCG42	HOVCC60	HMVAC92	HWGAF89	HHBEG78		19LfMJH 224	HPMJT61		HKAED89							

				63%	42	74
HIV 2.1.	HMMER 2.1.1	PFAM: Leucine Rich Repeat	PF00560	45.8	438	506
bla	stx.2	NG28.	sp Q9Z1P7 Q9Z1P7	25%	211	939
HIN 2.1	HMMER 2.1.1	PFAM: Ank repeat	PF00023	33.4	437	529
blas	blastx.14	similar to ankyrin of	gi 1136404 dbj BAA1	%88	200	724
		Chromatum vinosum. [Homo sapiens]	1489.1			
HIM 2.1.	MER 1	PFAM: Leucine Rich Repeat	PF00560	38.5	825	068
HM 2.1.	HMMER 2.1.1	PFAM: PWWP domain	PF00855	36.6	248	400
HM 2.1.	MER I	PFAM: PWWP domain	PF00855	68.3	5	226
HMIN 2.1.1	AER	PFAM: Leucine Rich Repeat	PF00560	36.4	235	303
blastx.2	λ.2	hypothetical protein	pir T27632 T27632	20%	82	504
		ZC518.3a - Caenorhabditis elegans		9%59	256	9/9
HMMER 2.1.1	ÆR	PFAM: B-box zinc finger.	PF00643	37	615	740
blast	x.14	(AF156271) RING finger	gi 5114351 gb AAD4	43%	612	755
		protein terf [Homo	0286.1	%09	382	486
		sapiens]		35%	528 502	611 525
blas	blastx.2	hypothetical protein DKFZp434N1511.1 - human (fragment)	pir T46316 T46316	100%	386	562
HM 2.1.	HMMER 2.1.1	PFAM: Ank repeat	PF00023	149.2	591	689

758	245	245	245	245	245	9//	245	245	764	764	245	245	218	758	245	764	215	764	245	716	245	770	245	764	758	245	764	764	245	764	764
585	9	9	9	9	9	582	9	9	585	597	42	9	9	582	9	009	9	585	9	585	9	585	9	582	009	9	585	579	9	582	009
41%	35%	35%	35%	37%	28%	32%	30%	36%	33%	33%	27%	28%	29%	37%	28%	34%	30%	33%	28%	40%	30%	32%	27%	31%	32%	28%	30%	30%	28%	32%	30%
gi 1841966 gb AAB4	7551.1					**		,					-					•		-											
ankyrin [Rattus	norvegicus]																														
blastx.14																															
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755	353	245	176	245	353	152	764	350	551	455	557	353	353	350	350	350	476	353	449	551	350	350	350	461	353	464	350	455	452	353	473
585	201	78	009	66	291	9	597	291	441	390	459	291	270	291	276	291	387	291	333	444	291	291	288	369	291	342	291	387	387	276	369
31%	27%	28%	28%	30%	57%	32%	30%	55%	40%	54%	36%	52%	39%	20%	48%	55%	46%	47%	35%	33%	20%	45%	47%	35%	47%	34%	20%	47%	20%	38%	34%
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353 464 557 350 350 347 449 656 656 653	799 1279 1024 1018 1024 1024	341	205 244 350 266
297 453 387 291 270 291 387 387 597 585	695 572 620 581 704 578	75	77 62 270 216
52% 3 3 3 3 3 3 3 3 3 3 3 3 8 % 8 4 4 4 2 8 8 % 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	186.2 98% 30% 26% 30% 22%	63.9	52.7 65% 59% 70%
,	PF00806 gi 1944416 dbj BAA1 9665.1	PF00635 gi 3135314 gb AAC7 8798.1	PF01217 gi 4929709 gb AAD3 4115.1 AF151878_1
	PFAM: Pumilio-family RNA binding domains (aka PUM-HD, Pumilio homology domain) similar to D.melanogaster pumilio protein (S22026): 1	PFAM: MSP (Major sperm protein) domain (AF053356) ORF3,splicevariant_b Homo sapiens]	PFAM: Clathrin adaptor complex small chain (AF151878) CGI-120 protein [Homo sapiens]
	HMMER 2.1.1 blastx.14	HMMER 2.1.1 blastx.14	HMMER 2.1.1 blastx.14
	187	188	189
	918008	919169	924850
	HODF103	HWHHR02	HSVBQ03

606	1022	1310	1119	413	1199	416	890	221	1070	153	402	632	499	239	239	416	242	233	215	197	248	653
754	924	882 759	718	171	1077	222	798	189	1008	88	4	45	383	63	63	63	63	63	63	126	150	162
55%	95.1	999	38%	60.7	63%	40%	61%	72%	38%	13.7	36%	%29	926	37%	33%	28%	30%	26%	767	35%	42.3	99%
sp 014593 RFXK_H UMAN	PF00023	gi 3820618 gb AAC6 9884.1	pir JC4385 JC4385	PF00595	gi[2996196 gb AAC0	8436.1				PF00023	sp Q9V583 Q9V583	sp Q9Y2V6 Q9Y2V6									PF00023	pir T42691 T42691
DNA-BINDING PROTEIN RFXANK.	PFAM: Ank repeat	(AF094761) Rfxank [Mus musculus]	LIM protein - rat	PFAM: PDZ domain (Also known as DHR or GLGF).	(AF053367) carboxyl	terminal LIM domain	protein [Mus musculus]			PFAM: Ank repeat	CG8809 PROTEIN.	HYPOTHETICAL 92.9	KDA PROTEIN.							The second secon	PFAM: Ank repeat	hypothetical protein DKFZn434D2328 1 -
blastx.2	HMMER 2.1.1	blastx.14	blastx.2	HMMER 2.1.1	blastx.14					HMMER 1.8	blastx.2	blastx.2									HMMER 2.1.1	blastx.2
190	314		191	315						192		193									316	194
1153914	963625		975382	948607						964579		1197348									863287	1152423
HSLCQ10	HSLCQ10		HKACQ38	НКАСО38					22	HE9GZ52		HSYBD55									HSYBD55	HTAJM37

				human (fragment)		35%	63	638
						35%	15	653
						34%	. 12	629
					- 3	33%	27	644
						33%	18	629
						32%	12	909
						28%	3	644
						34%	6	641
						30%	9	611
						29%	09	641
						29%	126	644
						29%	81	895
						38%	6	368
HTAJM37	911599	317	HMMER 2.1.1	PFAM: Ank repeat	PF00023	135	551	649
НЅЪЈН63	941120	195	HMMER 2.1.1	PFAM: Leucine Rich Repeat	PF00560	46.8	258	329
			blastx.2	Toll-like receptor 2	gb AAC34133.1	30%	180	1193
				Homo sapiens]		38%	75	82
HNNAG23	967549	318	HMMER 1.8	PFAM: LIM domain containing proteins	PF00412	53.96	375	548
	41.1.		blastx.14	mutant sterol regulatory	gi 841318 gb AAA85	%02	354	575
				element binding protein-2	718.1	40%	695	628
						45%	277	309
						33%	226	288
HYAAL21	943135	197	HMMER 2.1.1	PFAM: Leucine Rich Repeat	PF00560	37	449	517
			blastx.2	Leucine-rich-repeat	sp AAF66828 AAF66	30%	224	784
_				protein lrrA.	828	27%	212	802
						30%	308	754
HPBCF69	946469	198	HMMER	PFAM: Lencine Rich	PF00560	619	448	377
	12121	001	1	TITLE TORONTO	20000 11	7:10	2	

			2.1.1	Repeat				
			blastx.2	(AF126540) slit protein	gb AAD26567.1 AF1	37%	21	431
				[Drosophila melanogaster]	26540_1	31%	39	431
						32%	385	477
HWDAE40	947007	199	HMMER 2.1.1	PFAM: Leucine Rich Repeat	PF00560	132.1	586	1056
			blastx.2		SpiQ9WUG5 Q9WU	33%	250	804
					, <u>S</u>	33%	802	1332
						32%	692	1335
						79%	802	1701
						29%	307	1161
						30%	250	726
						79%	625	1092
						30%	402	1167
						31%	402	1086
						34%	493	906
21						29%	586	1020
						78%	421	870
						29%	901	1344
						29%	781	1161
						30%	499	873
						32%	604	939
						767	409	198
						79%	493	948
						34%	625	933
						27%	724	1086
						28%	748	1020
						31%	280	540
						79%	355	654
						37%	349	513
HUVHH77	948377	200	HMMER 2.1.1	PFAM: Leucine Rich Reneat	PF00560	54.6	807	884
_	_		7:1:7	1 Tropodit				

57% 264 920 45% 928 1416 30% 342 917	15.48 452 646	94% 2 823	92.9 273 605	91% 228 599	100% 718 741	20	23	29	14	14				36% 14 355		35% 23 346	90.7 280 378	37% 1 459	33% 10 483	31% 1 459	30% 1 456	33% 1 459
28461.1 AF1	7	AA91143 BAA9		gi[4877759]gb AAD3 91		 sp Q9VAU5 Q9VAU 49		75	33	38		35	35	36	37	35			4611.1 33		3(- 33
(AF169677) leucine-rich gb AAF repeat transmembrane 69677_1 protein FLRT3 [Homo sapiens]	PFAM: Double-stranded PF00035 RNA binding motif	CDNA FLJ20399 FIS, sp B/ CLONE KAT00581. 1143	PFAM: MAGE family PF01454	(AF124440) MAGE gi 48	tumor antigen D1 [Homo 1421	11 PROTEIN.	5				,	,					PFAM: Ank repeat PF00023	alt. ankyrin (variant 2.2) gi 74				-
blastx.2	201 HMMER 1.8	blastx.2	202 HMMER 2.1.1	blastx.14		203 blastx.2				-							319 HMMER 2.1.1	blastx.14				
	966870 20		951526 20			1152262 20											948288 3					
	HTLIT03		HUJDA09			HTEPU67		2	32								HTEPU67					

441 459 444 456 453 462 462 456 456 441 441 441 459 456	535	559	435	411	2	194
1 1 1 31 31 1 1 1 76 94	332	77	343	247	463	529
32% 30% 30% 28% 27% 27% 27% 28% 28% 28% 28%	69.7	100%	30.4	41%	109.4	48%
	PF00550	gi 2576345 gb AAC0 5814.1	PF00023	gij747710 emb CAA3 4611.1	PF01945	gi 5824388 emb CAA 93858.2
	PFAM: Phosphopantetheine attachment site	(AC002400) Acyl carrier protein, Mitochondrial (ACP) (5partial) [Homo sapiens]	PFAM: Ank repeat	alt. ankyrin (variant 2.2) [Homo sapiens]	PFAM: Domain of unknown function	similarity to 35.1KD hypothetical yeast protein 1 1 yk452e10.3 comes from this gene; cDNA EST yk452e10.5 comes
	HMMER 2.1.1	blastx.14	HMMER 2.1.1	blastx.14	HMMER 2.1.1	blastx.14
	204		320		321	
	952928		917406		954878	
	HULFJ52		HTEPV02		HTHBT91	

	993	318	299	325	497	1433	333	551	719	722	722	432	513	495	504	441	462	429	441	117
	112 1165	220	39	242	321	219	244	381	81	381	183	13	7	25	13	13	13	13	19	19
	%99 12%	43.1	64%	. 35.64	27.21	%98	25.5	194	%98	. 33%	24%	73%	36%	33%	31%	34%	36%	35%	30%	42.7
	pir B40858 B40858	PF00023	sp BAB01630 BAB0 1630	PF00023	PF00412	pir T34532 T34532	PF00855	PF00412	pir[JC2324]JC2324			pir T42691 T42691								PF00023
from t	GA-binding protein beta chain form 1 - mouse	PFAM: Ank repeat	Unnamed portein product.	PFAM: Ank repeat	PFAM: LIM domain containing proteins	hypothetical protein DKFZp434B1517.1 - human (fragment)	PFAM: PWWP domain	PFAM: LIM domain containing proteins	LIM protein - human			hypothetical protein	DKFZp434D2328.1 -	human (fragment)						PFAM: Ank repeat
	blastx.2	HIMMER 2.1.1	blastx.2	HMMER 1.8	HMMER 1.8	blastx.2	HMMER 2.1.1	HMMER 2.1.1	blastx.2			blastx.2								HMMER 2.1.1
	207	322	208	324	209		210	211				212	·		_					325
	1164631	813110	491273	880424	970481		958139	945350				1153890				=				661111
	HFVIH16	HFVIH16	HTJAB35	HTJAB35	HRABP94	-	HWAGC08	HRDET35				HGBIA24								HGBIA24

368	671	1148	170		1484	613	571	628	546	540	540	540	540	519	543	540	345	345	342	345	348	354	92	1322	1268	1601
327	594	351	06		289	191	209	518	454	337	364	364	364	364	367	349	247	256	256	259	256	256	3	78	177	1023
13.05	88	34%	31.6		%66	%56	33%	29%	71.2	41%	45%	37%	40%	40%	37%	34%	51%	43%	51%	37%	38%	33%	31.5	61%	35%	42%
PF00023	PF00560	gb AAC18782.1	PF00642		sp BAA90945 BAA9	0945			PF00023	gi 2447128 gb AAC9	6986.1												PF00023	sp Q9VUX2 Q9VUX	2	
PFAM: Ank repeat	PFAM: Leucine Rich Repeat	prolargin [Homo sapiens]	PFAM: Zinc finger C-x8-	C-x5-C-x3-H type (and similar).	CDNA FLJ20093 FIS,	CLONE COL04263.			PFAM: Ank repeat	contains 10 ankyrin-like	repeats; similar to human	1 [Paramecium bursaria	Chlorella virus 1]										PFAM: Ank repeat	CG5841 PROTEIN.		
HMMER 1.8	HMMER 2.1.1	blastx.2	HMMER	2.1.1	blastx.2				HMMER 2.1.1	blastx.14													HMMER 2.1.1	blastx.2		
213	214		215		216				326					·- <u>-</u>									327	218		
921596	964153		966226		1197927				910435														795268	1197928		
HTTHF21	HWHJZ40		HJMBN52		HUFCN47				HUFCN47			35											HHEUC31	HUSAL47		

	1/3332										_			_										J 11				
914 701 1589	548	422	410	422	308	404	404	422	404	410	404	404	404	422	401	404	410	404	422	410	404	407	410	521	521	521	512	521
177 270 1470	447	45	30	39	39	24	18	39	39	39	54	39	30	42	24	108	54	45	57	126	39	165	303	429	429	450	429	447
28% 29% 38%	116.8	37%	36%	32%	37%	767	31%	30%	30%	767	35%	30%	28%	76%	31%	31%	27%	31%	30%	30%	29%	27%	41%	51%	38%	54%	42%	44%
	PF00023	gi[710551 gb AAB01	605.1																						-			
	PFAM: Ank repeat	ankyrin 3 [Mus musculus]	٠							•																		
	HMMER 2.1.1	blastx.14														•												
	328																											
	211607																,											
	HUSAL47																											

521	512	527	521	518	521	299	605	521	512	545	512	512	575	521	575	512	809	9065	089	482	384	267	.267	871
447	424	432	450	450	432	516	522	429	450	438	444	429	498	411	525	459	516	507	393	384	286	46	115	096
44%	46%	37%	20%	47%	43%	35%	32%	32%	42%	27%	39%	78%	34%	73%	47%	44%	32%	35%	46%	37.1	36.8	69.5	59.2	83.8
																			sp(Q9UDM3 Q9UD M3	PF00023	PF00023	PF00855	PF00790	PF00023
																			WUGSC:H_DJ1035002.1 PROTEIN (FRAGMENT).	PFAM: Ank repeat	PFAM: Ank repeat	PFAM: PWWP domain	PFAM: VHS domain	PFAM: Ank repeat
								-											blastx.2	HMMER 2.1.1	HMMER 2.1.1	HMMER 2.1.1	HMMER 2.1.1	HMMER
						·													219	329	330	221	222	223
																			1153892	766126	966135	974684	689811	954681
															37				HHFGD38	HHFGD38	HVA0G11	HUVDR03	HUDAE29	HIBCJ89

			2.1.1					
			blastx.14	(AL034408) dJ710L4.2 (similar to	gi 4490506 emb CAB 38778.1	%86	1791	1204
				MYOTUBULARIN- RELATED PROTEIN) Homo saniensl				
HIBCJ89	963279	331	HMMER 2.1.1	PFAM: Ank repeat	PF00023	83.8	982	1071
			blastx.14	(AL034408) dJ710L4.2	gi 4490506 emb CAB	%66	151	738
				(similar to MYOTUBULARIN-	38778.1			
			—	RELATED PROTEIN) [Homo sapiens]				**
HIBEG40	504158	224	HMMER 2.1.1	PFAM: Ank repeat	PF00023	36.5	206	304
HWBEG33	1195806	225	blastx.2	hypothetical protein DKFZp586M2121.1 - human (fragment)	pir T46507 T46507	%66	114	1784
HWBEG33	702070	332	HMMER 2.1.1	PFAM: Ank repeat	PF00023	34	388	486
HWHKD22	1150878	226	blastx.2	RFXANK.	sp Q9Z205 Q9Z205	73%	15	308
HWHKD22	963626	333	HMMER 2.1.1	PFAM: Ank repeat	PF00023	55.2	308	406
			blastx.14	(AF094761) Rfxank [Mus musculus]	gi 3820618 gb AAC6 9884.1	72%	182	499
HSLF041	765497	227	HMMER 2.1.1	PFAM: Molybdenum cofactor biosynthesis protein	PF00994	139.6	1.1	249
HE9SE46	944511	228	HMMER 1.8	PFAM: Low-density lipoprotein receptor domain class A	PF00057	37.51	208	621

504 651 558	803	1027	698	563	1897	2249	371	1563	281	410	383	344	749	752	699	1020	561	549	399	450
61 499 484	321	482	597	462	1793	2199	303	1519	219	9	9	93	138	1111	349	922	454	289	289	376
33% 43% 36%	141.6	31%	31%	35%	37%	47%	43%	53%	43.4	43%	34%	40%	172.9	100%	8.06	45%	36%	24%	32%	26%
sp O43278 O43278	PF01852	pir A49678 A49678	gi 861029 emb CAA6	1011.1					PF00023	pir T12477 T12477			PF01852	sp Q9UKL6 Q9UKL6	PF00784	gi 3877186 emb CAA	91469.1			
HEPATOCYTE GROWTH FACTOR ACTIVATOR INHIBITOR	PFAM: START domain	GTPase-activating protein rhoGAP - human (fragment)	SH3 domain binding	protein [Mus musculus]					PFAM: Ank repeat	hypothetical protein	DKFZp564L0862.1 -	human (fragment)	PFAM: START domain	PHOSPHATIDYLCHOLI NE TRANSFER PROTEIN.	PFAM: Domain in Myosin and Kinesin Tails	Similarity to myosin;	cDNA EST yk249a4.5	comes from 1 1 gene;	cDNA EST yk470b4.5	comes from this gene;
blastx.2	HMMER 2.1.1	blastx.2	blastx.14						HMMER 2.1.1	blastx.2			HMMER 2.1.1	blastx.2	HMMER 2.1.1	blastx.14	-			
	229	230	334						231				232		233					
	864276	1227138	1056330						810433				921175		932448					
	HTLDW37	HWAFG54	HWAFG54						525 HKAFS73				HTXJD74		HSIGQ50					

906	. 726	162	159	145	575		965	726	104	578	•	<i>LL9</i>	€.		464	-	383	681
733	, 565	115	40	95	411		201	619	51	405		33			629		691	719
. 25%	27%	26%	25%	47%	84.8		44%	44%	38%	74.77		40%			75		%66	%69
	*		-		PF01018 ·		pir B72418 B72418			PF00089		sp Q9QY82 Q9QY82		•	PF01134		gi 4680645 gb AAD2	7712.1 AF132937_1
cDNA EST yk249a4		- 3			PFAM: GTP1/OBG	family	conserved hypothetical	protein - Thermotoga	maritima (strain MSB8)	PFAM: Trypsin		MOSAIC SERINE	PROTEASE	EPITHELIASIN.	PFAM: Glucose inhibited	division protein A	(AF132937) CGI-02	protein [Homo sapiens]
					HMMER	2.1.1	blastx.2			HMMER	1.8	blastx.2			HMMER	2.1.1	blastx.14	-
		•			234					235					236			i.e
			p2		932607			•	,	971537				•	946862			
					HWWDY45 932607					HINSMB24					HWLOU63		:	

Table 2 further characterizes certain encoded polypeptides of the invention, by providing the results of comparisons to protein and protein family databases. The first column provides a unique clone identifier, "Clone ID NO:", corresponding to a cDNA clone disclosed in Table 1A. The second column provides the unique contig identifier, "Contig ID:" which allows correlation with the information in Table 1A. The third column provides the sequence identifier, "SEQ ID NO:", for the contig polynucleotide sequences. The fourth column provides the analysis method by which the homology/identity disclosed in the Table was determined. The fifth column provides a description of the PFAM/NR hit identified by each analysis. Column six provides the accession number of the PFAM/NR hit disclosed in the fifth column. Column seven, score/percent identity, provides a quality score or the percent identity, of the hit disclosed in column five. Comparisons were made between polypeptides encoded by polynucleotides of the invention and a non-redundant protein database (herein referred to as "NR"), or a database of protein families (herein referred to as "PFAM"), as described below.

[45] The NR database, which comprises the NBRF PIR database, the NCBI GenPept database, and the SIB SwissProt and TrEMBL databases, was made non-redundant using the computer program nrdb2 (Warren Gish, Washington University in Saint Louis). Each of the polynucleotides shown in Table 1A, column 3 (e.g., SEQ ID NO:X or the 'Query' sequence) was used to search against the NR database. The computer program BLASTX was used to compare a 6-frame translation of the Query sequence to the NR database (for information about the BLASTX algorithm please see Altshul et al., J. Mol. Biol. 215:403-410 (1990); and Gish and States, Nat. Genet. 3:266-272 (1993). A description of the sequence that is most similar to the Query sequence (the highest scoring 'Subject') is shown in column five of Table 2 and the database accession number for that sequence is provided in column six. The highest scoring 'Subject' is reported in Table 2 if (a) the estimated probability that the match occurred by chance alone is less than 1.0e-07, and (b) the match was not to a known repetitive element. BLASTX returns alignments of short polypeptide segments of the Query and Subject sequences which share a high degree of similarity; these segments are known as High-Scoring Segment Pairs or HSPs. Table 2 reports the degree of similarity between the Query and the Subject for each HSP as a percent identity in Column 7. The percent identity is determined by dividing the number of exact matches between the two aligned sequences in the HSP, dividing by the number of Query amino acids in the HSP

and multiplying by 100. The polynucleotides of SEQ ID NO:X which encode the polypeptide sequence that generates an HSP are delineated by columns 8 and 9 of Table 2.

- [46] The PFAM database, PFAM version 2.1, (Sonnhammer et al., Nucl. Acids Res., 26:320-322, 1998)) consists of a series of multiple sequence alignments; one alignment for each protein family. Each multiple sequence alignment is converted into a probability model called a Hidden Markov Model, or HMM, that represents the position-specific variation among the sequences that make up the multiple sequence alignment (see, e.g., Durbin et al., Biological sequence analysis: probabilistic models of proteins and nucleic acids, Cambridge University Press, 1998 for the theory of HMMs). The program HMMER version 1.8 (Sean Eddy, Washington University in Saint Louis) was used to compare the predicted protein sequence for each Query sequence (SEQ ID NO:Y in Table 1A) to each of the HMMs derived from PFAM version 2.1. A HMM derived from PFAM version 2.1 was said to be a significant match to a polypeptide of the invention if the score returned by HMMER 1.8 was greater than 0.8 times the HMMER 1.8 score obtained with the most distantly related known member of that protein family. The description of the PFAM family which shares a significant match with a polypeptide of the invention is listed in column 5 of Table 2, and the database accession number of the PFAM hit is provided in column 6. Column 7 provides the score returned by HMMER version 1.8 for the alignment. Columns 8 and 9 delineate the polynucleotides of SEQ ID NO:X which encode the polypeptide sequence which show a significant match to a PFAM protein family.
- [47] As mentioned, columns 8 and 9 in Table 2, "NT From" and "NT To", delineate the polynucleotides of "SEQ ID NO:X" that encode a polypeptide having a significant match to the PFAM/NR database as disclosed in the fifth column. In one embodiment, the invention provides a protein comprising, or alternatively consisting of, a polypeptide encoded by the polynucleotides of SEQ ID NO:X delineated in columns 8 and 9 of Table 2. Also provided are polynucleotides encoding such proteins, and the complementary strand thereto.
- [48] The nucleotide sequence SEQ ID NO:X and the translated SEQ ID NO:Y are sufficiently accurate and otherwise suitable for a variety of uses well known in the art and described further below. For instance, the nucleotide sequences of SEQ ID NO:X are useful for designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the cDNA contained in Clone ID NO:Z. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling

immediate applications in chromosome mapping, linkage analysis, tissue identification and/or typing, and a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y may be used to generate antibodies which bind specifically to these polypeptides, or fragments thereof, and/or to the polypeptides encoded by the cDNA clones identified in, for example, Table 1A.

- [49] Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).
- [50] Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X, and a predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing cDNA Clone ID NO:Z (deposited with the ATCC on October 5, 2000, and receiving ATCC designation numbers PTA 2574 and PTA 2575; deposited with the ATCC on January 5, 2001, and having depositor reference numbers TS-1, TS-2, AC-1, and AC-2; and/or as set forth, for example, in Table 1A, 6 and 7). The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. Further, techniques known in the art can be used to verify the nucleotide sequences of SEQ ID NO:X.
- [51] The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

RACE Protocol For Recovery of Full-Length Genes

[52] Partial cDNA clones can be made full-length by utilizing the rapid amplification of cDNA ends (RACE) procedure described in Frohman, M.A., et al., Proc. Nat'l. Acad.

Sci. USA, 85:8998-9002 (1988). A cDNA clone missing either the 5' or 3' end can be reconstructed to include the absent base pairs extending to the translational start or stop codon, respectively. In some cases, cDNAs are missing the start codon of translation, The following briefly describes a modification of this original 5' RACE procedure. Poly A+ or total RNA is reverse transcribed with Superscript II (Gibco/BRL) and an antisense or complementary primer specific to the cDNA sequence. The primer is removed from the reaction with a Microcon Concentrator (Amicon). The first-strand cDNA is then tailed with dATP and terminal deoxynucleotide transferase (Gibco/BRL). Thus, an anchor sequence is produced which is needed for PCR amplification. The second strand is synthesized from the dA-tail in PCR buffer, Tag DNA polymerase (Perkin-Elmer Cetus), an oligo-dT primer containing three adjacent restriction sites (XhoI, SalI and ClaI) at the 5' end and a primer containing just these restriction sites. This double-stranded cDNA is PCR amplified for 40 cycles with the same primers as well as a nested cDNA-specific antisense primer. The PCR products are size-separated on an ethidium bromide-agarose gel and the region of gel containing cDNA products the predicted size of missing protein-coding DNA is removed. cDNA is purified from the agarose with the Magic PCR Prep kit (Promega), restriction digested with XhoI or SalI, and ligated to a plasmid such as pBluescript SKII (Stratagene) at XhoI and EcoRV sites. This DNA is transformed into bacteria and the plasmid clones sequenced to identify the correct protein-coding inserts. Correct 5' ends are confirmed by comparing this sequence with the putatively identified homologue and overlap with the partial cDNA clone. Similar methods known in the art and/or commercial kits are used to amplify and recover 3' ends.

[53] Several quality-controlled kits are commercially available for purchase. Similar reagents and methods to those above are supplied in kit form from Gibco/BRL for both 5' and 3' RACE for recovery of full length genes. A second kit is available from Clontech which is a modification of a related technique, SLIC (single-stranded ligation to single-stranded cDNA), developed by Dumas et al., Nucleic Acids Res., 19:5227-32 (1991). The major differences in procedure are that the RNA is alkaline hydrolyzed after reverse transcription and RNA ligase is used to join a restriction site-containing anchor primer to the first-strand cDNA. This obviates the necessity for the dA-tailing reaction which results in a polyT stretch that is difficult to sequence past.

[54] An alternative to generating 5' or 3' cDNA from RNA is to use cDNA library double-stranded DNA. An asymmetric PCR-amplified antisense cDNA strand is

synthesized with an antisense cDNA-specific primer and a plasmid-anchored primer. These primers are removed and a symmetric PCR reaction is performed with a nested cDNA-specific antisense primer and the plasmid-anchored primer.

RNA Ligase Protocol For Generating The 5' or 3' End Sequences To Obtain Full Length Genes

Once a gene of interest is identified, several methods are available for the [55] identification of the 5' or 3' portions of the gene which may not be present in the original cDNA plasmid. These methods include, but are not limited to, filter probing, clone enrichment using specific probes and protocols similar and identical to 5' and 3' RACE. While the full length gene may be present in the library and can be identified by probing, a useful method for generating the 5' or 3' end is to use the existing sequence information from the original cDNA to generate the missing information. A method similar to 5' RACE is available for generating the missing 5' end of a desired full-length gene. (This method was published by Fromont-Racine et al., Nucleic Acids Res., 21(7):1683-1684 (1993)). Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcript and a primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest, is used to PCR amplify the 5' portion of the desired full length gene which may then be sequenced and used to generate the full length gene. This method starts with total RNA isolated from the desired source, poly A RNA may be used but is not a prerequisite for this procedure. The RNA preparation may then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase if used is then inactivated and the RNA is treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase. This modified RNA preparation can then be used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction can then be used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the relevant gene.

[56] The present invention also relates to vectors or plasmids which include such DNA sequences, as well as the use of the DNA sequences. The material deposited with the ATCC (deposited with the ATCC on October 5, 2000, and receiving ATCC designation numbers PTA 2574 and PTA 2575; deposited with the ATCC on January 5, 2001, and receiving ATCC designation numbers TS-1, TS-2, AC-1, and AC-2; and/or as set forth, for example, in Table 1A, Table 6, or Table 7) is a mixture of cDNA clones derived from a variety of human tissue and cloned in either a plasmid vector or a phage vector, as described, for example, in Table 7. These deposits are referred to as "the deposits" herein. The tissues from which some of the clones were derived are listed in Table 7, and the vector in which the corresponding cDNA is contained is also indicated in Table 7. The deposited material includes cDNA clones corresponding to SEQ ID NO:X described, for example, in Table 1A (Clone ID NO:Z). A clone which is isolatable from the ATCC Deposits by use of a sequence listed as SEQ ID NO:X, may include the entire coding region of a human gene or in other cases such clone may include a substantial portion of the coding region of a human gene. Furthermore, although the sequence listing may in some instances list only a portion of the DNA sequence in a clone included in the ATCC Deposits, it is well within the ability of one skilled in the art to sequence the DNA included in a clone contained in the ATCC Deposits by use of a sequence (or portion thereof) described in, for example Tables 1Aor 2 by procedures hereinafter further described, and others apparent to those skilled in the art.

- [57] Also provided in Table 7 is the name of the vector which contains the cDNA clone. Each vector is routinely used in the art. The following additional information is provided for convenience.
- [58] Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., *Nucleic Acids Res. 16*:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., *Nucleic Acids Res. 17*:9494 (1989)) and pBK (Alting-Mees, M. A. et al., *Strategies 5*:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Phagemid pBS may be excised from the Lambda Zap and Uni-Zap XR vectors, and phagemid pBK may be excised from the Zap Express vector. Both phagemids may be transformed into *E. coli* strain XL-1 Blue, also available from Stratagene.

[59] Vectors pSport1, pCMVSport 1.0, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. See, for instance, Gruber, C. E., et al., *Focus 15:*59- (1993). Vector lafmid BA (Bento Soares, Columbia University, New York, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR[®]2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. See, for instance, Clark, J. M., *Nuc. Acids Res. 16:*9677-9686 (1988) and Mead, D. *et al.*, *Bio/Technology 9:* (1991).

- [60] The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or the deposited clone (Clone ID NO:Z). The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.
- Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X or the complement thereof, polypeptides encoded by genes corresponding to SEQ ID NO:X or the complement thereof, and/or the cDNA contained in Clone ID NO:Z, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.
- [62] The polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.
- [63] The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often

advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

[64] The polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the polypeptides of the present invention in methods which are well known in the art.

[65] The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or the cDNA sequence contained in Clone ID NO:Z. The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X or a complement thereof, a polypeptide encoded by the cDNA contained in Clone ID NO:Z, and/or the polypeptide sequence encoded by a nucleotide sequence in SEQ ID NO:B as defined in column 6 of Table 1B. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, a polypeptide encoded by the cDNA contained in Clone ID NO:Z, and/or a polypeptide sequence encoded by a nucleotide sequence in SEO ID NO:B as defined in column 6 of Table 1B are also encompassed by the invention. The present invention further encompasses a polynucleotide comprising, or alternatively consisting of, the complement of the nucleic acid sequence of SEQ ID NO:X, a nucleic acid sequence encoding a polypeptide encoded by the complement of the nucleic acid sequence of SEQ ID NO:X, and/or the cDNA contained in Clone ID NO:Z.

[66] Moreover, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in Table 1B column 6, or any combination thereof. Additional, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in Table 1B column 6, or any

combination thereof. In further embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in Table 1B, column 6, and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1B, column 5). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in Table 1B, column 6, and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1B, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in Table 1B, column 6, and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (see Table 1B, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides and polypeptides are also encompassed by the invention.

[67] Further, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in column 6 of Table 1B which correspond to the same Clone ID NO:Z (see Table 1B, column 1), or any combination thereof. Additional, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in column 6 of Table 1B which correspond to the same Clone ID NO:Z (see Table 1B, column 1), or any combination thereof. In further embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1B which correspond to the same Clone ID NO:Z (see Table 1B, column 1) and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1B, column 5). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1B which correspond to the same Clone ID NO:Z (see Table 1B, column 1) and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1B, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated

in column 6 of Table 1B which correspond to the same Clone ID NO:Z (see Table 1B, column 1) and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (see Table 1B, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides and polypeptides are also encompassed by the invention.

Further, representative examples of polynucleotides of the invention comprise, or [68] alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in column 6 of Table 1B which correspond to the same contig sequence identifier SEQ ID NO:X (see Table 1B, column 2), or any combination thereof. Additional, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in column 6 of Table 1B which correspond to the same contig sequence identifer SEQ ID NO:X (see Table 1B, column 2), or any combination thereof. In further embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1B which correspond to the same contig sequence identifer SEQ ID NO:X (see Table 1B, column 2) and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1B, column 5). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1B which correspond to the same contig sequence identifer SEQ ID NO:X (see Table 1B, column 2) and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1B, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in column 6 of Table 1B which correspond to the same contig sequence identifer SEQ ID NO:X (see Table 1B, column 2) and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (See Table 1B, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides and polypeptides are also encompassed by the invention.

Moreover, representative examples of polynucleotides of the invention comprise, [69] or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in the same row of Table 1B column 6, or any combination thereof. Additional, representative examples of polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in the same row of Table 1B column 6, or any combination thereof. In preferred embodiments, the polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the complementary strand(s) of the sequences delineated in the same row of Table 1B column 6, wherein sequentially delineated sequences in the table (i.e. corresponding to those exons located closest to each other) are directly contiguous in a 5' to 3' orientation. In further embodiments, above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in the same row of Table 1B, column 6, and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1B, column 5). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in the same row of Table 1B, column 6, and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1B, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated in the same row of Table 1B, column 6, and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (see Table 1B, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

[70] In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in column 6 of Table 1B, and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1B, column 2) or fragments or variants thereof. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in column 6 of Table 1B which correspond to the same Clone ID NO:Z (see Table 1B, column 1), and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1A or 1B) or fragments or variants thereof. In preferred embodiments, the delineated sequence(s) and polynucleotide sequence of SEQ ID NO:X correspond to the same Clone ID NO:Z. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

- In further specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more of the sequences delineated in the same row of column 6 of Table 1B, and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1A or 1B) or fragments or variants thereof. In preferred embodiments, the delineated sequence(s) and polynucleotide sequence of SEQ ID NO:X correspond to the same row of column 6 of Table 1B. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.
- In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B and the 5' 10 polynucleotides of the sequence of SEQ ID NO:X are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.
- [74] In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B and the 5' 10 polynucleotides of a fragment or variant of the sequence of SEQ ID NO:X are directly contiguous Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent

hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of the sequence of SEQ ID NO:X and the 5' 10 polynucleotides of the sequence of one of the sequences delineated in column 6 of Table 1B are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of a fragment or variant of the sequence of SEQ ID NO:X and the 5' 10 polynucleotides of the sequence of one of the sequences delineated in column 6 of Table 1B are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides, are also encompassed by the invention.

[77] In further specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B and the 5' 10 polynucleotides of another sequence in column 6 are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization

conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B and the 5' 10 polynucleotides of another sequence in column 6 corresponding to the same Clone ID NO:Z (see Table 1B, column 1) are directly contiguous. Nucleic acids which hybridize to the complement of these 20 lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence in which the 3' 10 polynucleotides of one sequence in column 6 corresponding to the same contig sequence identifer SEQ ID NO:X (see Table 1B, column 2) are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

[80] In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B and the 5' 10 polynucleotides of another sequence in column 6 corresponding to the same row are directly contiguous. In preferred embodiments, the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B is directly contiguous with the 5' 10 polynucleotides of the next

sequential exon delineated in Table 1B, column 6. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

[81] Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. Accordingly, for each contig sequence (SEQ ID NO:X) listed in the fourth column of Table 1A, preferably excluded are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 and the final nucleotide minus 15 of SEQ ID NO:X, b is an integer of 15 to the final nucleotide of SEQ ID NO:X, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:X, and where b is greater than or equal to a + 14. More specifically, preferably excluded are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a and b are integers as defined in columns 4 and 5, respectively, of Table 3. In specific embodiments, the polynucleotides of the invention do not consist of at least one, two, three, four, five, ten, or more of the specific polynucleotide sequences referenced by the Genbank Accession No. as disclosed in column 6 of Table 3 (including for example, published sequence in connection with a particular BAC clone). In further embodiments, preferably excluded from the invention are the specific polynucleotide sequence(s) contained in the clones corresponding to at least one, two, three, four, five, ten, or more of the available material having the accession numbers identified in the sixth column of this Table (including for example, the actual sequence contained in an identified BAC clone). In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example. All references available through these accessions are hereby incorporated by reference in their entirety.

TABLE 3

	SEQ				
	ID				
Clone ID	NO:	Contig	EST Dis		
NO: Z	_X_	ID:	Range of a	Range of b	Accession #'s
HFRBN59	11	1106393	1 - 432	15 - 446	
HE2KJ64	12	906019	1 - 913	15 - 927	AA164661, AI962647, AA164645,
					AA096157, T77033, AI656439, T69166,
					Z44826, R55232, AA164782, AA496160,
					AA247800, AA091213, AC020570, and
TIL CDY 700	10	1170,000	1 1000	15 1000	AC020570.
HAGDV32	13	1178626	1 - 1889	15 - 1903	AL049128, AA398117, R54194, AA394218, AA081522, AA247129, and AF061936.
HLICC37	14	856958	1 - 624	15 - 638	AA203242, AL365356, AL365356, and
nLices/	14	030930	1 - 024	13 - 036	AL365356.
HBGBU96	15	1121900	1 - 649	15 - 663	111111111111111111111111111111111111111
HAJCQ63	16	823850	1 - 777	15 - 791	AA902808, AI002049, AF082556, and
,					AF082557.
HLMMV66	17	1153903	1 - 738	15 - 752	AW084519, AI244442, AA614014, AI808637,
}		1			AA903338, AI342240, AI962752, AI717991,
				,	AW139714, AI921541, AI660761, D20168,
					AW138271, AI219797, AI041118, F19235,
		!			AI798637, F16699, AI201892, T29020,
					AI792451, AI054048, AW393736,
•	}				AW393737, AA748165, AA918804,
	}				AA811883, AW271140, AW207518,
XII XI/A DOO	10	100.6200	1 510	15 522	AA525796, AW393733, and D30758.
HLWAR08	18	1096389	1 - 519	15 - 533 15 - 830	AA078617, AJ133128, and AF160798.
HBGTT76	19	1152327	1 - 816	15 - 830	AI284640, AL046409, AW419262, AI963720, AI613280, AW193265, AI431303, AI305766,
ļ	ļ			ļ	A1801482, A1334443, AA581903, AL119691,
ļ]	}		ļ	A1345654, A1270117, A1281881, AA587604,
					AW327868, AL037683, AW439558,
İ	ĺ				AI708009, AL045053, AW303196, AI133164,
	}				AW274349, AW301350, AL041690,
	<u> </u>				AW408717, AI110770, AA569471,
					AL046205, AA610491, AI076616, AL138455,
	[AW021583, AI754253, AW265393,
1	ĺ	1			AL138265, AW276827, AI312309,
ŧ					AW028429, AA491814, AI350211,
]	ļ			AL044940, AA490183, AI754658, AA526787,
	Ì	ļ			AI064864, AW238278, AI696962, AA720702,
	1	Į			AW438643, AI799642, AI270559, F36273,
	ĺ				AA521323, AI345681, AI345675, AI610159,
ł	}			}	A1969436, AA394271, A1679782, AL042753,
	1]		}	AI469968, AW088846, AI619997, AA468022, AI064952, AW406162, AI754336, AI305547,
}]			}	A1064952, AW406162, A1754336, A1505547, A1341664, AW268300, A1623720, A1473943,
		[AI341004, AW 208300, AI023720, AI473943, AI345518, AA164251, AA521399, AI149478,
	1	[AI798473, AW265009, AW407578,
1	ł	1			AI192631, AI289067, AI133102, AI633390,
	<u> </u>]]	AI368256, AI471481, AI249997, AA491284,
1	}])	AA613345, AI821271, AI061334, AW083402,
1					AI732865, AW004911, AW270270,
	1				AW073470, AI688846, AL079645,
1	}				AW410400, AA857486, AI871722,
}]	ļ		AW029038, AA584201, AI570261, AI355206,
			L	L	AW302013, AW193432, AA533333,

AI053672, AW162049, AI962050, AI345157, AI929531, AI307201, AA649642, AI801591, AI624142, AW103758, AI744995, AW148792, AW021207, AA551552, AI375710, AA126051, AA126035, AL134972, AW304584, AW131249, AI370094, AI339850, AA507824, H71429, AI379719, AI801600, AW338086, AI344844, AI298710, AW301809, AI559705, AA468131, AI370074, AL038785, AI567076, AA846935, AI805363, AI340453, AI457397, AA877817, AW167372, AI499503, AA623002, AI246119, AI696955, AI286356, AI246796, AA610271, AI792287, AI653886, AA503258, AW261871, AI368745, AA847499, AI376100, AL042853, AW410354, AI921649, AI281697, AI053790, AA584145, AL120687, AA533036, AI587565, AA587256, AI538433, AA713815, AA621858, C75026, AL046457, AL048626, AI564496, AA488746, AI085719, AI358571, AI499134, AL038703, AW339568, AL121235, AW166815, AI623898, AI148277, AA493471, AA631507, AA503473, R24887, AL135405, AW269488, AW276435, AL038705, AA703820, AL038474, AA780515, AA502155, AI674873, AI635818, AI499938, AI625244, AI688928, AI538852, AI357288, AA806796, AA758934, AI890918, AI918421, AI017415, AA084070, AI281903, AI860020, AW406447, AA491831, AL009179, AP000513, D83989, AF015147, AF077058, AC006138, M37551, AF015151, X75335, U57006, U57007, AF015149. AF015156, AF015157, U18394, X54180, U18391, U18392, X55925, U57005, X54176, U57009, U18396, Z97205, X54181, X54178, X53550, U18395, U57008, AL096775, I51997, U18393, AL022238, X55926, U18399, Z97200, Z70688, AJ229041, AF015148, X54175, X55931, X55924, U18387, AC006251, AC005251, X54179, U18390, AL034419, AC005486, AC008249, AF015153, U67221, Z98742, U18398, U67801, AC005914, AP000455, AC002526, AL035455, AL049829, AC006476, AC003102, AC005211, AC004477, X55923, AC004895, AL021392, AL050342, AC005962, AC004217, AC005154, AC005180, AC005859, AL110292, X55927, AF134726, AF027390, AC010175, AC006004, AC004890, U18400, AC004234, X74558, AL049795, U91326, AL132985, U67231, AC005144, AC005921, AC007226, AC005005, AC010197, AC010200, AF015160, AC005829, AC005296, AF085913, X55922, Z22650, AC005664, AL031257, X55932, AC006511, AC016026, S43650, U12580, AC007043, AL022163, AC006088, AC004987, AC007682, U67233.

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HHSDM19	84	956045	1 - 1835	15 - 1849	AA461035, AI672417, AI922318, AA455380, AA207253, AW073828, AI656613, AI015897, AA081097, AI354270, AA223211, AA187149, AA207246, W07268, N79769, AA453976, AA765863, AF114029, AA456020, AA382282, AI287653, AA648749, R41461, AI535793, AI536006, D61645, AA992075, R58462, AA564978, AA204953, AA232403, AA382281, AA209404, Z64530, and Z64529.
HDTIT49	85	956917	1 - 841	15 - 855	AL138392, AA427786, T70793, AL138393, and AB033051.
HTLGW19	86	1163072	1 - 1948	15 - 1962	AA523383, AI742620, AI761443, AI952075, AW058000, AW051868, AI003892, AI963321, AI125092, AA740995, AA505650, AA458975, AA459191, AW292537, R36885,

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HE9TA54	88	960253	1 - 1847	15 - 1861	A1978973, AW369076, AW370703,
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HHESP66	91	1154641	1 - 1036	15 - 1050	AI125852, AA259012, AA224099, AW087456, AA326934, AA326933, AC005188, AC006291, and AF028722.
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ННГМН12	94	969324	1 - 3882	15 - 3896	AI096627, AI750041, AI589918, AI870013, AI431911, AW071873, AI567485, AI492558, AW082735, AI493768, AI971206, AW068564, AI494149, AA158252, AI422826, AI363488, AI460100, AW104306, AA100840, AI755276, AA476207, AI992015, AW026405, AI190217, AA678831, AI376927, AI738539, AI439206, AA037160, AI418906, AI361483, AI038534, AA877117, AI356122, AA425180,

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HIBCN93	96	973679	1 - 1492	15 - 1506	AI056401, AW072652, AI885072, AW205916, AI879122, AI885524, N34233, AI953626, AI768363, AI500165, AI887770, AI651798, AA393235, N35730, AW297174, AI802927, AW271854, R56346, AA249118, AI377463, AW070857, AI824909, N53527, AA948310, AI206861, AA572777, AA570002, AA814424, N25635, AA255602, AI478500, N25539, AA634056, H07018, N28490, AA416965, N34136, N34013, AA902860, AA255576, AW029208, AI690664, AI198003, H99526, H05467, N49189, AI190195, AI864484, N30121, H99763, AI024777, AA379362, AA262183,

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HSLCB60 HSLFG64 HTPFX16 HWAER24	132 133 134 135	1193050 1228145 974296 934693	1 - 1537 1 - 2976 1 - 470 1 - 2706	15 - 1551 15 - 2990 15 - 484 15 - 2720	AC003119, AC007021, AC005154, AP000952, AC002072, AC006251, AL031447, AC005768, AF123462, and AC007565. AW062391, and A14709. AI526148, and L29346. AI073560, AI201459, AA527879, AA700382, AA151678, AA777810, AA286925,
HSLFG64 HTPFX16	133 134	1228145 974296	1 - 2976 1 - 470	15 - 2990 15 - 484	AL031447, AC005768, AF123462, and AC007565. AW062391, and A14709. AI526148, and L29346. AI073560, AI201459, AA527879, AA700382, AA151678, AA777810, AA286925,
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		1			H43300, AI222506, W24708, AA492470,
					AA579885, T47922, T47923, AA348753,
	1			ľ	T05906, AA455850, H43299, AW292633,
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					AA772416, AA147480, N92391, AA378964,
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		AL036227, AL036163, AL037047,
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		AL036152, D80043, D80219, T48598,
		AL036900, T23656, AL037178, AA514190,
		AL048425, AW451416, AL039555, D80227,
		AL037085, Z25782, AL036174, AL036953,
		D80240, AL036808, D80134, AL037569,
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		D80210, AW450376, H00072, D59619,
	1	AL036268, AL038043, C14227, AL044447,
	1	D80193, AL037077, D80196, AI557751,
		AL037002, AL039417, D80168, Z25783,
		AU357002, AU39417, D80108, Z25783, AW135155, D59927, D80366, AU036858,
	j	C75259, C14389, AL037016, AL036229,
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HIBEG40	224	504158	1 - 389	15 - 403	AA351313, and AA351814.
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HWBEG33	225	1195806	1 - 2298	15 - 2312	AA195189, AI872284, AI970956, AA195225,
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					AI354761, AW014492, AI696473, AI815233,
1	1				AA205305, AA811626, AA418029,
					AA808125, N70046, AI825035, AI371756,
					AI680927, AI632886, H39858, AW302373,
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HSLFO41	227	765497	1 - 336	15 - 350	
HE9SE46	228	944511	1 - 2126	15 - 2140	AA195155, AI268255, AW419341, AI824127, AI797143, AI300923, AI292148, AI703401, AI268439, AI292153, AW137704, AI831208, R35403, AW137395, AI379414, AI379109, AI277432, AA057594, AI432198, AI300400, AI300976, AI300968, AW138254, AA862254, AA978306, AA136742, AA187853, AA411758, AW139302, AA418285, AI697655, AA136133, AI299234, W44727, AW135673, AA933000, AA195026, AW134622, AI336837, AI214619, AW388217, AA406572, AI342824, AW388179, AI222659, AI378218, AW370464, AA878171, AA825160, R25577, AI871540, AW388239, AA985538, AI468745, AW206391, T10355, AA424539, T75128, AA418322, R05548, AA976873, AA363106, F11165, H09479, AA114288, F12796, AA346579, AA112328, AA917973, Z42588, R18424, AA424606, AA781256, AA625611, AI633662, AA331566, N90086, AA055551, C18803, AI695403, AI741622, AW378385, AA995638, N63577, F05973, AA455944, AA190723, AA904239, AA436288, AI752009, and AI305270.
HTLDW37	229	864276	1 - 1104	15 - 1118	AW025605, AW025604, AA862436, AA375852, AI732328, AA371876, AI033275, and AB014607.
HWAFG54	230	1227138	1 - 3744	15 - 3758	AI760827, AW408019, AI253155, AI349366, AI356482, AA814034, AW075920, AW407984, AI760691, AA251937, AI766650, AA352825, AA243541, AI934100, AA352840, H72208, H72106, AW193021, AL035530, and AW664438.
HKAFS73	231	810433	1 - 396	15 - 410	N80779.
HTXJD74	232	921175	1 - 1097	15 - 1111	AA030013, W04200, T96295, AA348729, AW369326, AI817745, AI902506, AI740457, AA303510, AI902496, AW298344, AA843339, AI902495, AI902509, W19941, AW452907, AI902510, AI610042, AF151638, AF114430, U21660, Z50026, AF151639, AF040261, AF114436, Z50024, AF114437, AF114431, AF114434, AF114432, AF114433, AF114435, and AF040266.
HSIGQ50	233	932448	1 - 1332	15 - 1346	AW361379, AA131062, AC015551, AC019214, and AC019214.
HWWDY4 5	234	932607	1 - 713	15 - 727	AA451973, AW044300, AI925874, AC002064, and AC006153.
HNSMB24	235	971537	1 - 671	15 - 685	AI978874, AI469095, AP001623, AP001623, AC015555, and AC015555.
HWLOU63	236	946862	1 - 709	15 - 723	AI074147, AI249752, AA573289, AI744674, AW081142, AI951269, AI560208, AI309528, AI097133, AI310351, AI222028, AW073286, AA121301, AI160271, AI991117, AW170797, AA278853, H47623, AA742972, AA864447, AI572193, AA173309, AW188877, H69345,

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AW372736, AI476011, AW372731,
AW372739, AW372742, AW372744, N22901,
AA769896, AW372786, AW372740,
AW388634, AW372738, AW372734,
AW372745, AW372735, AW372737, H69344,
and AF132937.

TABLE 4

Code	Description	Tissue	Organ	Cell Line	Disease	Vector
AR022	a_Heart	a_Heart				
AR023	a_Liver	a_Liver				
AR024	a_mammary gland	a_mammary gland				
AR025	a_Prostate	a_Prostate				
AR026	a_small intestine	a_small intestine				
AR027	a_Stomach	a_Stomach				
AR028	Blood B cells	· Blood B cells				
AR029	Blood B cells activated	Blood B cells				
		activated				
AR030	Blood B cells resting	Blood B cells			-	
		resting				
AR031	Blood T cells activated	Blood T cells	}	}		
		activated				
AR032	Blood T cells resting	Blood T cells resting				
AR033	brain	brain				
AR034	breast	breast				
AR035	breast cancer	breast cancer				
AR036	Cell Line CAOV3	Cell Line CAOV3				
AR037	cell line PA-1	cell line PA-1				
AR038	cell line transformed	cell line transformed				
AR039	colon	colon				
AR040	colon (9808co65R)	colon (9808co65R)				
AR041	colon (9809co15)	colon (9809co15)				
AR042	colon cancer	colon cancer				
AR043	colon cancer (9808co64R)	colon cancer (9808co64R)				
AR044	colon cancer 9809co14	colon cancer 9809co14				
AR045	corn clone 5	corn clone 5				
AR046	corn clone 6	corn clone 6				
AR047	corn clone2	corn clone2				
AR048	corn clone3	corn clone3			<u> </u>	
AR049	Corn Clone4	Corn Clone4				
AR050	Donor II B Cells 24hrs	Donor II B Cells 24hrs				
AR051	Donor II B Cells 72hrs	Donor II B Cells 72hrs				
AR052	Donor II B-Cells 24 hrs.	Donor II B-Cells 24 hrs.				
AR053	Donor II B-Cells 72hrs	Donor II B-Cells				
AR054	Donor II Resting B Cells	72hrs Donor II Resting B				
AR055	Heart	Cells Heart		- -	 	<u> </u>
AR056	Human Lung (clonetech)	Human Lung			 	1
		(clonetech)				
AR057	Human Mammary (clontech)	Human Mammary (clontech)				

AR058	Human Thymus	Human Thymus				
	(clonetech)	(clonetech)				
AR059	Jurkat (unstimulated)	Jurkat (unstimulated)				
AR060	Kidney	Kidney				
AR061	Liver	Liver				
AR062	Liver (Clontech)	Liver (Clontech)				
AR063	Lymphocytes chronic	Lymphocytes				
	lymphocytic leukaemia	chronic lymphocytic				
		leukaemia				
AR064	Lymphocytes diffuse large	Lymphocytes				
	B cell lymphoma	diffuse large B cell		1	1	
15065		lymphoma			<u> </u>	
AR065	Lymphocytes follicular	Lymphocytes	ļ			
A D O C C	lymphoma normal breast	follicular lymphoma				
AR066 AR067	Normal Ovarian	normal breast Normal Ovarian		 		
ARUO7	(4004901)	(4004901)				
AR068	Normal Ovary 9508G045	Normal Ovary	<u> </u>			
7111000	11011111111 07111 7 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3	9508G045				
AR069	Normal Ovary 9701G208	Normal Ovary			<u> </u>	
		9701G208				
AR070	Normal Ovary 9806G005	Normal Ovary				
		9806G005				
AR071	Ovarian Cancer	Ovarian Cancer				
AR072	Ovarian Cancer	Ovarian Cancer			İ	
	(9702G001)	(9702G001)			<u> </u>	
AR073	Ovarian Cancer	Ovarian Cancer				
170-1	(9707G029)	(9707G029)			 	
AR074	Ovarian Cancer (9804G011)	Ovarian Cancer (9804G011)				
AR075	Ovarian Cancer	Ovarian Cancer	<u> </u>	+	 	
AROIS	(9806G019)	(9806G019)	- 0			
AR076	Ovarian Cancer	Ovarian Cancer		1	<u> </u>	
	(9807G017)	(9807G017)				
AR077	Ovarian Cancer	Ovarian Cancer				
: !	(9809G001)	(9809G001)				
AR078	ovarian cancer 15799	ovarian cancer	-			
		15799			<u> </u>	
AR079	Ovarian Cancer	Ovarian Cancer				
	17717AID	17717AID			ļ	
AR080	Ovarian Cancer	Ovarian Cancer				
4 D001	4004664B1	4004664B1			 	
AR081	Ovarian Cancer 4005315A1	Ovarian Cancer			1	
AR082	ovarian cancer 94127303	4005315A1 ovarian cancer		 		
73002	0 varian cancer 9412/303	94127303				
AR083	Ovarian Cancer 96069304	Ovarian Cancer		 	 	
	,	96069304				
AR084	Ovarian Cancer 9707G029	Ovarian Cancer				
		9707G029				
AR085	Ovarian Cancer 9807G045	Ovarian Cancer				
		9807G045				<u> </u>

AR086	ovarian cancer 9809G001	ovarian cancer 9809G001				
AR087	Ovarian Cancer 9905C032RC	Ovarian Cancer 9905C032RC				
AR088	Ovarian cancer 9907 C00 3rd	Ovarian cancer 9907 C00 3rd				
AR089	Prostate	Prostate				
AR090	Prostate (clonetech)	Prostate (clonetech)				
AR091	prostate cancer	prostate cancer			1	
AR092	prostate cancer #15176	prostate cancer #15176				
AR093	prostate cancer #15509	prostate cancer #15509				
AR094	prostate cancer #15673	prostate cancer #15673				
AR095	Small Intestine (Clontech)	Small Intestine (Clontech)				
AR096	Spleen	Spleen				
AR097	Thymus T cells activated	Thymus T cells activated				
AR098	Thymus T cells resting	Thymus T cells resting				
AR099	Tonsil	Tonsil				
AR100	Tonsil geminal center	Tonsil geminal			1	
141100	centroblast	center centroblast	į		1	
AR101	Tonsil germinal center B	Tonsil germinal			 	
	cell	center B cell				
AR102	Tonsil lymph node	Tonsil lymph node				
AR103	Tonsil memory B cell	Tonsil memory B				
AR104	Whole Brain	Whole Brain				
AR105	Xenograft ES-2	Xenograft ES-2				
AR106	Xenograft SW626	Xenograft SW626				
H0002	Human Adult Heart	Human Adult Heart	Heart			Uni-ZAP XR
H0004	Human Adult Spleen	Human Adult Spleen	Spleen			Uni-ZAP XR
H0007	Human Cerebellum	Human Cerebellum	Brain			Uni-ZAP XR
H0008	Whole 6 Week Old Embryo					Uni-ZAP XR
H0009	Human Fetal Brain			1		Uni-ZAP XR
H0012	Human Fetal Kidney	Human Fetal Kidney	Kidney			Uni-ZAP XR
H0013	Human 8 Week Whole Embryo	Human 8 Week Old Embryo	Embryo			Uni-ZAP XR
H0014	Human Gall Bladder	Human Gall Bladder	Gall Bladder			Uni-ZAP XR
H0015	Human Gall Bladder, fraction II	Human Gall Bladder	Gall Bladder			Uni-ZAP XR
H0016	Human Greater Omentum	Human Greater Omentum	peritoneum			Uni-ZAP XR
H0018	Human Greater Omentum, fII remake	Human Greater Omentum	peritoneum			Uni-ZAP XR
H0023	Human Fetal Lung					Uni-ZAP XR
H0024	Human Fetal Lung III	Human Fetal Lung	Lung			Uni-ZAP XR

H0026	Namalwa Cells	Namalwa B-Cell Line, EBV immortalized				Lambda ZAP II
H0028	Human Old Ovary	Human Old Ovary	Ovary			pBluescript
H0030	Human Placenta					Uni-ZAP XR
H0031	Human Placenta	Human Placenta	Placenta			Uni-ZAP XR
H0032	Human Prostate	Human Prostate	Prostate			Uni-ZAP XR
H0033	Human Pituitary	Human Pituitary				Uni-ZAP XR
H0036	Human Adult Small Intestine	Human Adult Small Intestine	Small Int.			Uni-ZAP XR
H0037	Human Adult Small Intestine	Human Adult Small Intestine	Small Int.			pBluescript
H0038	Human Testes	Human Testes	Testis			Uni-ZAP XR
H0039	Human Pancreas Tumor	Human Pancreas Tumor	Pancreas		disease	Uni-ZAP XR
H0040	Human Testes Tumor	Human Testes Tumor	Testis		disease	Uni-ZAP XR
H0041	Human Fetal Bone	Human Fetal Bone	Bone			Uni-ZAP XR
H0042	Human Adult Pulmonary	Human Adult Pulmonary	Lung			Uni-ZAP XR
H0046	Human Endometrial Tumor	Human Endometrial Tumor	Uterus		disease	Uni-ZAP XR
H0047	Human Fetal Liver	Human Fetal Liver	Liver			Uni-ZAP XR
H0048	Human Pineal Gland	Human Pineal Gland				Uni-ZAP XR
H0050	Human Fetal Heart	Human Fetal Heart	Heart			Uni-ZAP XR
H0051	Human Hippocampus	Human Hippocampus	Brain			Uni-ZAP XR
H0052	Human Cerebellum	Human Cerebellum	Brain			Uni-ZAP XR
H0056	Human Umbilical Vein, Endo. remake	Human Umbilical Vein Endothelial Cells	Umbilical vein			Uni-ZAP XR
H0057	Human Fetal Spleen	Cons				Uni-ZAP XR
H0058	Human Thymus Tumor	Human Thymus Tumor	Thymus		disease	Lambda ZAP II
H0059	Human Uterine Cancer	Human Uterine Cancer	Uterus		disease	Lambda ZAP II
H0063	Human Thymus	Human Thymus	Thymus			Uni-ZAP XR
H0064	Human Right Hemisphere of Brain	Human Brain, right hemisphere	Brain			Uni-ZAP XR
H0068	Human Skin Tumor	Human Skin Tumor	Skin		disease	Uni-ZAP XR
H0069	Human Activated T-Cells	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0070	Human Pancreas	Human Pancreas	Pancreas			Uni-ZAP XR
H0071	Human Infant Adrenal Gland	Human Infant Adrenal Gland	Adrenal gland			Uni-ZAP XR
H0075	Human Activated T-Cells (II)	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0079	Human Whole 7 Week Old Embryo (II)	Human Whole 7 Week Old Embryo	Embryo			Uni-ZAP XR
H0081	Human Fetal Epithelium (Skin)	Human Fetal Skin	Skin			Uni-ZAP XR
H0083	HUMAN JURKAT MEMBRANE BOUND	Jurkat Cells				Uni-ZAP XR

	POLYSOMES					
H0085	Human Colon	Human Colon		1		Lambda ZAP II
H0086	Human epithelioid	Epithelioid	Sk Muscle		disease	Uni-ZAP XR
120000	sarcoma	Sarcoma, muscle	OK WIGGOID	1	discuse	OIN-22AI AIN
H0087	Human Thymus	Human Thymus				pBluescript
H0090	Human T-Cell Lymphoma	T-Cell Lymphoma	T-Cell		disease	Uni-ZAP XR
H0097	Human Adult Heart,	Human Adult Heart	Heart		discuso	pBluescript
110057	subtracted	Tuman Adult (teat	Heart			pDiaescript
H0098	Human Adult Liver, subtracted	Human Adult Liver	Liver			Uni-ZAP XR
H0099	Human Lung Cancer, subtracted	Human Lung Cancer	Lung			pBluescript
H0100	Human Whole Six Week Old Embryo	Human Whole Six Week Old Embryo	Embryo			Uni-ZAP XR
H0101	Human 7 Weeks Old Embryo, subtracted	Human Whole 7 Week Old Embryo	Embryo			Lambda ZAP II
H0102	Human Whole 6 Week Old Embryo (II), subt	Human Whole Six Week Old Embryo	Embryo			pBluescript
H0103	Human Fetal Brain, subtracted	Human Fetal Brain	Brain			Uni-ZAP XR
H0109	Human Macrophage, subtracted	Macrophage	Blood	Cell Line		pBluescript
H0111	Human Placenta, subtracted	Human Placenta	Placenta			pBluescript
H0116	Human Thymus Tumor, subtracted	Human Thymus Tumor	Thymus			pBluescript
H0119	Human Pediatric Kidney	Human Pediatric Kidney	Kidney			Uni-ZAP XR
H0122	Human Adult Skeletal Muscle	Human Skeletal Muscle	Sk Muscle			Uni-ZAP XR
H0123	Human Fetal Dura Mater	Human Fetal Dura Mater	Brain			Uni-ZAP XR
H0124	Human Rhabdomyosarcoma	Human Rhabdomyosarcoma	Sk Muscle		disease	Uni-ZAP XR
H0125	Cem cells cyclohexamide treated	Cyclohexamide Treated Cem, Jurkat, Raji, and Supt	Blood	Cell Line		Uni-ZAP XR
H0130	LNCAP untreated	LNCAP Cell Line	Prostate	Cell Line		Uni-ZAP XR
H0134	Raji Cells, cyclohexamide treated	Cyclohexamide Treated Cem, Jurkat, Raji, and Supt	Blood	Cell Line		Uni-ZAP XR
H0135	Human Synovial Sarcoma	Human Synovial Sarcoma	Synovium			Uni-ZAP XR
H0136	Supt Cells, cyclohexamide treated	Cyclohexamide Treated Cem, Jurkat, Raji, and Supt	Blood	Cell Line		Uni-ZAP XR
H0141	Activated T-Cells, 12 hrs.	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0144	Nine Week Old Early	9 Wk Old Early	Embryo			Uni-ZAP XR
	Stage Human	Stage Human]
H0149	7 Week Old Early Stage	Human Whole 7	Embryo			Uni-ZAP XR
	Human, subtracted	Week Old Embryo	W			
H0150	Human Epididymus	Epididymis	Testis			Uni-ZAP XR
H0151	Early Stage Human Liver	Human Fetal Liver	Liver	1		Uni-ZAP XR

H0152	Early Stage Human Liver, fract (II)	Human Fetal Liver	Liver		,	Uni-ZAP XR
H0155	Human Thymus, subtracted	Human Thymus Tumor	Thymus			pBluescript
H0156	Human Adrenal Gland Tumor	Human Adrenal Gland Tumor	Adrenal Gland		disease	Uni-ZAP XR
H0161	Activated T-Cells, 24 hrs., ligation 2	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0163	Human Synovium	Human Synovium	Synovium			Uni-ZAP XR
Н0164	Human Trachea Tumor	Human Trachea Tumor	Ţrachea		disease	Uni-ZAP XR
H0165	Human Prostate Cancer, Stage B2	Human Prostate Cancer, stage B2	Prostate		disease	Uni-ZAP XR
H0166	Human Prostate Cancer, Stage B2 fraction	Human Prostate Cancer, stage B2	Prostate		disease	Uni-ZAP XR
Н0169	Human Prostate Cancer, Stage C fraction	Human Prostate Cancer, stage C	Prostate		disease	Uni-ZAP XR
H0170	12 Week Old Early Stage Human	Twelve Week Old Early Stage Human	Embryo			Uni-ZAP XR
H0171	12 Week Old Early Stage Human, II	Twelve Week Old Early Stage Human	Embryo			Uni-ZAP XR
H0173	Human Cardiomyopathy, RNA remake	Human Cardiomyopathy	Heart		disease	Uni-ZAP XR
H0178	Human Fetal Brain	Human Fetal Brain	Brain			Uni-ZAP XR
H0179	Human Neutrophil	Human Neutrophil	Blood	Cell Line		Uni-ZAP XR
H0181	Human Primary Breast Cancer	Human Primary Breast Cancer	Breast		disease	Uni-ZAP XR
H0187	Resting T-Cell	T-Cells	Blood	Cell Line		Lambda ZAP II
H0188	Human Normal Breast	Human Normal Breast	Breast			Uni-ZAP XR
H0194	Human Cerebellum, subtracted	Human Cerebellum	Brain			pBluescript
H0196	Human Cardiomyopathy, subtracted	Human Cardiomyopathy	Heart			Uni-ZAP XR
H0197	Human Fetal Liver, subtracted	Human Fetal Liver	Liver			Uni-ZAP XR
H0198	Human Fetal Liver, subtracted, pos. clon	Human Fetal Liver	Liver			Uni-ZAP XR
H0199	Human Fetal Liver, subtracted, neg clone	Human Fetal Liver	Liver			Uni-ZAP XR
H0200	Human Greater Omentum, fract II remake,	Human Greater Omentum	peritoneum	•		Uni-ZAP XR
H0204	Human Colon Cancer, subtracted	Human Colon Cancer	Colon			pBluescript
H0207	LNCAP, differential expression	LNCAP Cell Line	Prostate	Cell Line		pBluescript
H0208	Early Stage Human Lung, subtracted	Human Fetal Lung	Lung			pBluescript
H0212	Human Prostate, subtracted	Human Prostate	Prostate			pBluescript
H0213	Human Pituitary, subtracted	Human Pituitary	-			Uni-ZAP XR
H0214	Raji cells, cyclohexamide	Cyclohexamide	Blood	Cell Line		pBluescript

		, 	<u></u>			7
	treated, subtracted	Treated Cem, Jurkat, Raji, and Supt			•	
H0216	Supt cells, cyclohexamide treated, subtracted	Cyclohexamide Treated Cem, Jurkat, Raji, and Supt	Blood	Cell Line		pBluescript
H0217	Supt cells, cyclohexamide treated, differentially expressed	Cyclohexamide Treated Cem, Jurkat, Raji, and Supt	Blood	Cell Line		pBluescript
H0225	Activated T-Cells, 12hrs, differentially expressed	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0228	C7MCF7 cell line, estrogen treated	C7MCF7 Cell Line, estrogen treated	Breast	Cell Line		Uni-ZAP XR
H0230	Human Cardiomyopathy, diff exp	Human Cardiomyopathy	Heart		disease	Uni-ZAP XR
H0231	Human Colon, subtraction	Human Colon				pBluescript
H0235	Human colon cancer, metaticized to liver, subtraction	Human Colon Cancer, metasticized to liver	Liver			pBluescript
H0239	Human Kidney Tumor	Human Kidney Tumor	Kidney		disease	Uni-ZAP XR
H0242	Human Fetal Heart, Differential (Fetal- Specific)	· Human Fetal Heart	Heart			pBluescript
H0244	Human 8 Week Whole Embryo, subtracted	Human 8 Week Old Embryo	Embryo			Uni-ZAP XR
H0246	Human Fetal Liver- Enzyme subtraction	Human Fetal Liver	Liver			Uni-ZAP XR
H0247	Human Membrane Bound Polysomes- Enzyme Subtraction	Human Membrane Bound Polysomes	Blood	Cell Line		Uni-ZAP XR
H0249	HE7, subtracted by hybridization with E7 cDNA	Human Whole 7 Week Old Embryo	Embryo			Uni-ZAP XR
H0250	Human Activated Monocytes	Human Monocytes				Uni-ZAP XR
H0251	Human Chondrosarcoma	Human Chondrosarcoma	Cartilage		disease	Uni-ZAP XR
H0252	Human Osteosarcoma	Human Osteosarcoma	Bone		disease	Uni-ZAP XR
H0253	Human adult testis, large inserts	Human Adult Testis	Testis			Uni-ZAP XR
H0254	Breast Lymph node cDNA library	Breast Lymph Node	Lymph Node			Uni-ZAP XR
H0255	breast lymph node CDNA library	Breast Lymph Node	Lymph Node			Lambda ZAP II
H0261	H. cerebellum, Enzyme subtracted	Human Cerebellum	Brain		•	Uni-ZAP XR
H0263	human colon cancer	Human Colon Cancer	Colon		disease	Lambda ZAP II
H0264	human tonsils	Human Tonsil	Tonsil			Uni-ZAP XR
H0265	Activated T-Cell (12hs)/Thiouridine labelledEco	T-Cells	Blood	Cell Line		Uni-ZAP XR

H0266	Human Microvascular Endothelial Cells, fract. A	HMEC	Vein	Cell Line	1	Lambda ZAP II
H0267	Human Microvascular Endothelial Cells, fract. B	HMEC	Vein	Cell Line		Lambda ZAP II
H0268	Human Umbilical Vein Endothelial Cells, fract. A	HUVE Cells	Umbilical vein	Cell Line		Lambda ZAP II
H0270	HPAS (human pancreas, subtracted)	Human Pancreas	Pancreas			Uni-ZAP XR
H0271	Human Neutrophil, Activated	Human Neutrophil - Activated	Blood	Cell Line		Uni-ZAP XR
H0272	HUMAN TONSILS, FRACTION 2	Human Tonsil	Tonsil			Uni-ZAP XR
H0282	HBGB"s differential consolidation	Human Primary Breast Cancer	Breast			Uni-ZAP XR
H0286	Human OB MG63 treated (10 nM E2) fraction I	Human Osteoblastoma MG63 cell line	Bone	Cell Line		Uni-ZAP XR
H0288	Human OB HOS control fraction I	Human Osteoblastoma HOS cell line	Bone	Cell Line		Uni-ZAP XR
H0290	Human OB HOS treated (1 nM E2) fraction I	Human Osteoblastoma HOS cell line	Bone	Cell Line		Uni-ZAP XR
H0292	Human OB HOS treated (10 nM E2) fraction I	Human Osteoblastoma HOS cell line	Bone	Cell Line		Uni-ZAP XR
H0294	Amniotic Cells - TNF induced	Amniotic Cells - TNF induced	Placenta	Cell Line		Uni-ZAP XR
H0295	Amniotic Cells - Primary Culture	Amniotic Cells - Primary Culture	Placenta	Cell Line		Uni-ZAP XR
H0298	HCBB"s differential consolidation	CAMA1Ee Cell Line	Breast	Cell Line		Uni-ZAP XR
H0305	CD34 positive cells (Cord Blood)	CD34 Positive Cells	Cord Blood			ZAP Express
Н0306	CD34 depleted Buffy Coat (Cord Blood)	CD34 Depleted Buffy Coat (Cord Blood)	Cord Blood			ZAP Express
H0309	Human Chronic Synovitis	Synovium, Chronic Synovitis/ Osteoarthritis	Synovium		disease	Uni-ZAP XR
H0316	HUMAN STOMACH	Human Stomach	Stomach			Uni-ZAP XR
H0318	HUMAN B CELL LYMPHOMA	Human B Cell Lymphoma	Lymph Node		disease	Uni-ZAP XR
H0320	Human frontal cortex	Human Frontal Cortex	Brain			Uni-ZAP XR
H0321	HUMAN SCHWANOMA	Schwanoma	Nerve		disease	Uni-ZAP XR
H0327	human corpus colosum	Human Corpus Callosum	Brain			Uni-ZAP XR
H0328	human ovarian cancer	Ovarian Cancer	Ovary		disease	Uni-ZAP XR
H0329	Dermatofibrosarcoma Protuberance	Dermatofibrosarcom a Protuberans	Skin		disease	Uni-ZAP XR
H0331	Hepatocellular Tumor	Hepatocellular Tumor	Liver		disease	Lambda ZAP II

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H0333	Hemangiopericytoma	Hemangiopericytom a	Blood yessel		disease	Lambda ZAP II
H0334	Kidney cancer	Kidney Cancer	Kidney		disease	Uni-ZAP XR
H0340	Corpus Callosum	Corpus Collosum- 93052				Uni-ZAP XR
H0341	Bone Marrow Cell Line (RS4;11)	Bone Marrow Cell Line RS4;11	Bone Marrow	Cell Line		Uni-ZAP XR
H0343	stomach cancer (human)	Stomach Cancer - 5383A (human)			disease	Uni-ZAP XR
H0346	Brain-medulloblastoma	Brain (Medulloblastoma)- 9405C006R	Brain		disease	Uni-ZAP XR
H0349	human adult liver cDNA library	Human Adult Liver	Liver			pCMVSport 1
H0350	Human Fetal Liver, mixed 10 & 14 week	Human Fetal Liver, mixed 10&14 Week	Liver			Uni-ZAP XR
H0351	Glioblastoma	Glioblastoma	Brain		disease	Uni-ZAP XR
H0352	wilm"s tumor	Wilm"s Tumor			disease	Uni-ZAP XR
H0354	Human Leukocytes	Human Leukocytes	Blood	Cell Line		pCMVSport 1
H0355	Human Liver	Human Liver, normal Adult				pCMVSport 1
H0356	Human Kidney	Human Kidney	Kidney			pCMVSport 1
Н0357	H. Normalized Fetal Liver, II	Human Fetal Liver	Liver			Uni-ZAP XR
H0361	Human rejected kidney	Human Rejected Kidney			disease	pBluescript
H0365	Osteoclastoma-normalized B	Human Osteoclastoma			disease	Uni-ZAP XR
H0369	H. Atrophic Endometrium	Atrophic Endometrium and myometrium				Uni-ZAP XR
H0370	H. Lymph node breast Cancer	Lymph node with Met. Breast Cancer			disease	Uni-ZAP XR
H0373	Human Heart	Human Adult Heart	Heart			pCMVSport 1
H0375	Human Lung	Human Lung				pCMVSport 1
H0379	Human Tongue, frac 1	Human Tongue				pSport1
H0380	Human Tongue, frac 2	Human Tongue				pSport1
H0383	Human Prostate BPH, re- excision	Human Prostate BPH				Uni-ZAP XR
H0392	H. Meningima, M1	Human Meningima	brain			pSport1
H0393	Fetal Liver, subtraction II	Human Fetal Liver	Liver			pBluescript
H0396	L1 Cell line	Redd-Sternberg cell				ZAP Express
H0399	Human Kidney Cortex, re- rescue	Human Kidney Cortex				Lambda ZAP II
H0400	Human Striatum Depression, re-rescue	Human Brain, Striatum Depression	Brain			Lambda ZAP II
H0402	CD34 depleted Buffy Coat (Cord Blood), re-excision	CD34 Depleted Buffy Coat (Cord Blood)	Cord Blood			ZAP Express
H0405	Human Pituitary, subtracted VI	Human Pituitary				pBluescript
H0408	Human kidney Cortex, subtracted	Human Kidney Cortex				pBluescript

H0409	H. Striatum Depression,	Human Brain,	Brain			pBluescript
	subtracted	Striatum Depression		<u> </u>		
H0411	H Female Bladder, Adult	Human Female Adult Bladder	Bladder			pSport1
H0412	Human umbilical vein endothelial cells, IL-4 induced	HUVE Cells	Umbilical vein	Cell Line		pSport1
H0413	Human Umbilical Vein Endothelial Cells, uninduced	HUVE Cells	Umbilical vein	Cell Line		pSport1
H0415	H. Ovarian Tumor, II, OV5232	Ovarian Tumor, OV5232	Ovary		disease	pCMVSport 2.0
H0416	Human Neutrophils, Activated, re-excision	Human Neutrophil - Activated	Blood	Cell Line		pBluescript
H0419	Bone Cancer, re-excision	Bone Cancer				Uni-ZAP XR
H0421	Human Bone Marrow, re- excision	Bone Marrow				pBluescript
H0422	T-Cell PHA 16 hrs	T-Cells	Blood	Cell Line		pSport1
H0423	T-Cell PHA 24 hrs	T-Cells	Blood	Cell Line		pSport1
H0424	Human Pituitary, subt IX	Human Pituitary				pBluescript
H0427	Human Adipose	Human Adipose, left hiplipoma				pSport1
H0428	Human Ovary	Human Ovary Tumor	Ovary			pSport1
H0431	H. Kidney Medulla, re- excision	Kidney medulla	Kidney			pBluescript
H0433	Human Umbilical Vein Endothelial cells, frac B, re-excision	HUVE Cells	Umbilical vein	Cell Line		pBluescript
H0434	Human Brain, striatum, re-excision	Human Brain, Striatum				pBluescript
H0435	Ovarian Tumor 10-3-95	Ovarian Tumor, OV350721	Ovary			pCMVSport 2.0
H0436	Resting T-Cell Library,II	T-Cells	Blood	Cell Line		pSport1
H0437	H Umbilical Vein Endothelial Cells, frac A, re-excision	HUVE Cells	Umbilical vein	Cell Line		Lambda ZAP II
H0438	H. Whole Brain #2, re- excision	Human Whole Brain #2				ZAP Express
H0441	H. Kidney Cortex, subtracted	Kidney cortex	Kidney			pBluescript
H0444	Spleen metastic melanoma	Spleen, Metastic malignant melanoma	Spleen		disease	pSport1
H0445	Spleen, Chronic lymphocytic leukemia	Human Spleen, CLL	Spleen		disease	pSport1
H0455	H. Striatum Depression, subt	Human Brain, Striatum Depression	Brain			pBluescript
H0457	Human Eosinophils	Human Eosinophils				pSport1
H0459	CD34+cells, II, FRACTION 2	CD34 positive cells				pCMVSport 2.0
H0478	Salivary Gland, Lib 2	Human Salivary Gland	Salivary gland			pSport1

H0483	Breast Cancer cell line,	Breast Cancer Cell				pSport1
	MDA 36	line, MDA 36				
H0484	Breast Cancer Cell line,	Breast Cancer Cell	Ì			pSport1
	angiogenic	line, Angiogenic,				}
		36T3				
H0485	Hodgkin"s Lymphoma I	Hodgkin"s			disease	pCMVSport 2.0
		Lymphoma I		<u> </u>		
H0486	Hodgkin"s Lymphoma II	Hodgkin"s			disease	pCMVSport 2.0
		Lymphoma II		<u></u>		
H0487	Human Tonsils, lib I	Human Tonsils				pCMVSport 2.0
H0488	Human Tonsils, Lib 2	Human Tonsils				pCMVSport 2.0
H0489	Crohn"s Disease	Ileum	Intestine		disease	pSport1
H0492	HL-60, RA 4h, Subtracted	HL-60 Cells, RA	Blood	Cell Line		Uni-ZAP XR
		stimulated for 4H				
H0494	Keratinocyte	Keratinocyte				pCMVSport 2.0
H0497	HEL cell line	HEL cell line		HEL		pSport1
				92.1.7		
H0506	Ulcerative Colitis	Colon	Colon			pSport1
H0509	Liver, Hepatoma	Human Liver,	Liver		disease	pCMVSport 3.0
~~~~		Hepatoma, patient 8				<u> </u>
H0510	Human Liver, normal	Human Liver,	Liver	[		pCMVSport 3.0
******		normal, Patient #8			******	
H0518	pBMC stimulated w/ poly	pBMC stimulated		}		pCMVSport 3.0
110710	I/C	with poly I/C				
H0519	NTERA2, control	NTERA2,				pCMVSport 3.0
		Teratocarcinoma				
110520	NEEDAGAAAA	cell line				6 41
H0520	NTERA2 + retinoic acid,	NTERA2, Teratocarcinoma		,		pSport1
	14 days	cell line				
H0521	Primary Dendritic Cells,	Primary Dendritic				pCMVSport 3.0
110521	lib 1	cells				pcwrv sport 3.0
H0522	Primary Dendritic	Primary Dendritic				pCMVSport 3.0
110322	cells, frac 2	cells				pelvi v Sport 3.0
H0525	PCR, pBMC I/C treated	pBMC stimulated				PCRII
110525	Tere, powie i/e treated	with poly I/C	:			1 CKII
H0527	Human Liver,	Human Liver,	Liver			pSport1
110327	normal,CapFinder	normal, Patient #8	Eivoi			poporti
H0529	Myoloid Progenitor Cell	TF-1 Cell Line;				pCMVSport 3.0
	Line	Myoloid progenitor				Politispoissis
		cell line				
H0530	Human Dermal	Human Dermal				pSport1
	Endothelial	Endothelial Cells;		l i		1
	Cells,untreated	untreated				
H0538	Merkel Cells	Merkel cells	Lymph node			pSport1
H0539	Pancreas Islet Cell Tumor	Pancreas Islet Cell	Pancreas		disease	pSport1
		Tumour				
H0542	T Cell helper I	Helper T cell				pCMVSport 3.0
H0543	T cell helper II	Helper T cell		1		pCMVSport 3.0
H0544	Human endometrial	Human endometrial				pCMVSport 3.0
	stromal cells	stromal cells	_	[		
H0545	Human endometrial	Human endometrial				pCMVSport 3.0
	stromal cells-treated with	stromal cells-treated	1	1		1 *

	progesterone	with proge		1		T
H0546	Human endometrial	Human endometrial				pCMVSport 3.0
110540	stromal cells-treated with	stromal cells-treated				pCM v Sport 3.0
	estradiol	with estra				
H0547	NTERA2 teratocarcinoma	NTERA2,	<del> </del>	<u> </u>		-01
<b>n</b> 0347	cell line+retinoic acid (14	· · · · · · · · · · · · · · · · · · ·				pSport1
	· '	Teratocarcinoma	β			
TT05.40	days)	cell line		<del></del>		1
H0549	H. Epididiymus, caput &	Human				Uni-ZAP XR
	corpus	Epididiymus, caput	ļ	}		
		and corpus				-
H0550	H. Epididiymus, cauda	Human				Uni-ZAP XR
		Epididiymus, cauda				
H0551	Human Thymus Stromal	Human Thymus	ļ.	1		pCMVSport 3.0
	Cells	Stromal Cells				
H0553	Human Placenta	Human Placenta				pCMVSport 3.0
H0555	Rejected Kidney, lib 4	Human Rejected Kidney	Kidney		disease	pCMVSport 3.0
H0556	Activated T-	T-Cells	Blood	Cell Line		Uni-ZAP XR
	cell(12h)/Thiouridine-re-					
	excision		İ			
H0559	HL-60, PMA 4H, re-	HL-60 Cells, PMA	Blood	Cell Line		Uni-ZAP XR
	excision	stimulated 4H	2.000			0.11.22.11.21.11
H0560	KMH2	KMH2				pCMVSport 3.0
H0561	L428	L428	<del>                                     </del>			pCMVSport 3.0
H0562	Human Fetal Brain,	Human Fetal Brain				
110302	normalized c5-11-26	Human Fetal Diam				pCMVSport 2.0
H0563	Human Fetal Brain,	Human Fetal Brain				pCMVSport 2.0
	normalized 50021F					
H0570	Human Fetal Brain,	Human Fetal Brain				pCMVSport 2.0
	normalized C500H		1			P
H0572	Human Fetal Brain,	Human Fetal Brain				pCMVSport 2.0
	normalized AC5002		Ì			point Sport 2.0
H0574	Hepatocellular Tumor; re-	Hepatocellular	Liver		disease	Lambda ZAP II
11057.	excision	Tumor	Liver	Į	discase	Lamoda Z/M H
H0575	Human Adult	Human Adult	Lung			Uni-ZAP XR
110575	Pulmonary;re-excision	Pulmonary	Lung			UIII-ZAF AK
H0576	Resting T-Cell; re-	T-Cells	Pland	Cell Line	,	Lambda ZAP II
110570	excision	1-Cells	Blood	Cell Line		Lamoda ZAP II
H0579	Pericardium	Pericardium	Heart			pSport1
H0580	Dendritic cells, pooled	Pooled dendritic				pCMVSport 3.0
	1	cells				
H0581	Human Bone Marrow,	Human Bone	Bone Marrow			pCMVSport 3.0
	treated	Marrow				pent opens.
H0583	B Cell lymphoma	B Cell Lymphoma	B Cell		disease	pCMVSport 3.0
H0584	Activated T-cells, 24	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
11030-	hrs,re-excision	101111100 1-00113	Diood	Con Line		Jin-Zati Ait
H0585	Activated T-Cells,12	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
110505	hrs,re-excision	1 Touvaiou 1 "Cons	l Diood	Con Line		OIII-ZAI AK
H0586	Healing groin wound, 6.5	healing groin			diass	DCMVC20
77000			groin		disease	pCMVSport 3.0
	hours post incision	wound, 6.5 hours	1		1	1
110507	Hasling quair 1.7.5	post incision - 2/	l		1.	C) AVC 122
H0587	Healing groin wound; 7.5 hours post incision	Groin-2/19/97	groin		disease	pCMVSport 3.0
	Hours post mersion	L	<u> </u>	L		

H0589	CD34 positive cells (cord blood),re-ex	CD34 Positive Cells	Cord Blood			ZAP Express
H0590	Human adult small intestine, re-excision	Human Adult Small Intestine	Small Int.			Uni-ZAP XR
H0591	Human T-cell lymphoma;re-excision	T-Cell Lymphoma	T-Cell		disease	Uni-ZAP XR
H0592	Healing groin wound - zero hr post-incision (control)	HGS wound healing project; abdomen			disease	pCMVSport 3.0
H0593	Olfactory epithelium;nasalcavity	Olfactory epithelium from roof of left nasal cacit				pCMVSport 3.0
H0594	Human Lung Cancer;re- excision	Human Lung Cancer	Lung		disease	Lambda ZAP II
H0595	Stomach cancer (human);re-excision	Stomach Cancer - 5383A (human)			disease	Uni-ZAP XR
H0596	Human Colon Cancer;re- excision	Human Colon Cancer	Colon			Lambda ZAP II
H0597	Human Colon; re-excision	Human Colon				Lambda ZAP II
H0598	Human Stomach;re- excision	Human Stomach	Stomach			Uni-ZAP XR
H0599	Human Adult Heart;re- excision	Human Adult Heart	Heart			Uni-ZAP XR
H0600	Healing Abdomen wound;70&90 min post incision	Abdomen			disease	pCMVSport 3.0
H0606	Human Primary Breast Cancer;re-excision	Human Primary Breast Cancer	Breast		disease	Uni-ZAP XR
H0608	H. Leukocytes, control	H.Leukocytes				pCMVSport 1
H0613	H.Leukocytes, normalized cot 5B	H.Leukocytes				pCMVSport 1
H0614	H. Leukocytes, normalized cot 500 A	H.Leukocytes				pCMVSport 1
H0615	Human Ovarian Cancer Reexcision	Ovarian Cancer	Ovary		disease	Uni-ZAP XR
H0616	Human Testes, Reexcision	Human Testes	Testis			Uni-ZAP XR
H0617	Human Primary Breast Cancer Reexcision	Human Primary Breast Cancer	Breast		disease	Uni-ZAP XR
H0618	Human Adult Testes, Large Inserts, Reexcision	Human Adult Testis	Testis			Uni-ZAP XR
H0619	Fetal Heart	Human Fetal Heart	Heart			Uni-ZAP XR
H0620	Human Fetal Kidney; Reexcision	Human Fetal Kidney	Kidney			Uni-ZAP XR
H0622	Human Pancreas Tumor; Reexcision	Human Pancreas - Tumor	Pancreas		disease	Uni-ZAP XR
H0623	Human Umbilical Vein; Reexcision	Human Umbilical Vein Endothelial Cells	Umbilical vein			Uni-ZAP XR
H0624	12 Week Early Stage Human II; Reexcision	Twelve Week Old Early Stage Human	Embryo			Uni-ZAP XR
H0625	Ku 812F Basophils Line	Ku 812F Basophils	<del></del>	l		pSport1
H0626	Saos2 Cells; Untreated	Saos2 Cell Line; Untreated				pSport1

H0627	Saos2 Cells; Vitamin D3 Treated	Saos2 Cell Line; Vitamin D3 Treated			,	pSport1
H0628	Human Pre-Differentiated Adipocytes	Human Pre- Differentiated Adipocytes				Uni-ZAP XR
H0631	Saos2, Dexamethosome Treated	Saos2 Cell Line; Dexamethosome Treated				pSport1
H0632	Hepatocellular Tumor;re- excision	Hepatocellular Tumor	Liver			Lambda ZAP II
H0633	Lung Carcinoma A549 TNFalpha activated	TNFalpha activated A549Lung Carcinoma			disease	pSport1
H0634	Human Testes Tumor, re- excision	Human Testes Tumor	Testis		disease	Uni-ZAP XR
H0635	Human Activated T-Cells, re-excision	Activated T-Cells	Blood	Cell Line		Uni-ZAP XR
H0637	Dendritic Cells From CD34 Cells	Dentritic cells from CD34 cells				pSport1
H0638	CD40 activated monocyte dendridic cells	CD40 activated monocyte dendridic cells				pSport1
H0641	LPS activated derived dendritic cells	LPS activated monocyte derived dendritic cells				pSport1
H0642	Hep G2 Cells, lambda library	Hep G2 Cells				Other
H0643	Hep G2 Cells, PCR library	Hep G2 Cells				Other
H0644	Human Placenta (re- excision)	Human Placenta	Placenta			Uni-ZAP XR
H0645	Fetal Heart, re-excision	Human Fetal Heart	Heart			Uni-ZAP XR
H0646	Lung, Cancer (4005313 A3): Invasive Poorly Differentiated Lung Adenocarcinoma,	Metastatic squamous cell lung carcinoma, poorly di				pSport1
Н0647	Lung, Cancer (4005163 B7): Invasive, Poorly Diff. Adenocarcinoma, Metastatic	Invasive poorly differentiated lung adenocarcinoma			disease	pSport1
H0648	Ovary, Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm, Low Malignant Pot	Papillary Cstic neoplasm of low malignant potentia			disease	pSport1
H0649	Lung, Normal: (4005313 B1)	Normal Lung				pSport1
H0650	B-Cells	B-Cells				pCMVSport 3.0
H0651	Ovary, Normal: (9805C040R)	Normal Ovary				pSport1
H0652	Lung, Normal: (4005313 B1)	Normal Lung				pSport1
H0653	Stromal Cells	Stromal Cells	-			pSport1
H0654	Lung, Cancer: (4005313 A3) Invasive Poorly-	Metastatic Squamous cell lung				Other

_	differentiated Metastatic lung adenoc	Carcinoma poorly dif			
H0656	B-cells (unstimulated)	B-cells (unstimulated)			pSport1
H0657	B-cells (stimulated)	B-cells (stimulated)			pSport1
H0658	Ovary, Cancer (9809C332): Poorly differentiated adenocarcinoma	9809C332- Poorly differentiate	Ovary & Fallopian Tubes	disease	pSport1
H0659	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma	Grade II Papillary Carcinoma, Ovary	Ovary	disease	pSport1
H0660	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma	Poorly differentiated carcinoma, ovary		disease	pSport1
H0661	Breast, Cancer: (4004943 A5)	Breast cancer		disease	pSport1
H0662	Breast, Normal: (4005522B2)	Normal Breast - #4005522(B2)	Breast		pSport1
H0663	Breast, Cancer: (4005522 A2)	Breast Cancer - #4005522(A2)	Breast	disease	pSport1
H0664	Breast, Cancer: (9806C012R)	Breast Cancer	Breast	disease	pSport1
H0665	Stromal cells 3.88	Stromal cells 3.88			pSport1
H0666	Ovary, Cancer: (4004332 A2)	Ovarian Cancer, Sample #4004332A2		disease	pSport1
H0667	Stromal cells(HBM3.18)	Stromal cell(HBM 3.18)			pSport1
H0668	stromal cell clone 2.5	stromal cell clone 2.5			pSport1
H0669	Breast, Cancer: (4005385 A2)	Breast Cancer (4005385A2)	Breast		pSport1
H0670	Ovary, Cancer(4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma	Ovarian Cancer - 4004650A3			pSport1
H0671	Breast, Cancer: (9802C02OE)	Breast Cancer- Sample # 9802C02OE			pSport1
H0672	Ovary, Cancer: (4004576 A8)	Ovarian Cancer(4004576A8)	Ovary		pSport1
H0673	Human Prostate Cancer, Stage B2; re-excision	Human Prostate Cancer, stage B2	Prostate		Uni-ZAP XR
H0674	Human Prostate Cancer, Stage C; re-excission	Human Prostate Cancer, stage C	Prostate		Uni-ZAP XR
H0675	Colon, Cancer: (9808C064R)	Colon Cancer 9808C064R			pCMVSport 3.0
H0676	Colon, Cancer: (9808C064R)-total RNA	Colon Cancer 9808C064R			pCMVSport 3.0
H0677	TNFR degenerate oligo	B-Cells			PCRII
H0682	Serous Papillary Adenocarcinoma	serous papillary adenocarcinoma			pCMVSport 3.0

		(0.00.000.000.000.000	]			T
*******	0 1 0 0 0	(9606G304SPA3B)				
H0683	Ovarian Serous Papillary	Serous papillary	}	}		pCMVSport 3.0
	Adenocarcinoma	adenocarcinoma,				
H0684	Corana Donillami	stage 3C (9804G01 Ovarian Cancer-	Ovaries			-CN (V/C+2/
HU004	Serous Papillary Adenocarcinoma	9810G606	Ovaries	•		pCMVSport 3.0
H0685	Adenocarcinoma of	Adenocarcinoma of				-CMD/S+2/
H0003	Ovary, Human Cell Line,	Ovary, Human Cell	}	1		pCMVSport 3.0
	# OVCAR-3	Line, # OVCAR-				)
H0686	Adenocarcinoma of	Adenocarcinoma of				pCMVSport 3.0
110000	Ovary, Human Cell Line	Ovary, Human Cell				pcivi v sport 3.0
	o ary, manian con Line	Line, # SW-626				i
H0687	Human normal	Human normal	Ovary			pCMVSport 3.0
	ovary(#9610G215)	ovary(#9610G215)		ļ		1
H0688	Human Ovarian	Human Ovarian				pCMVSport 3.0
	Cancer(#9807G017)	cancer(#9807G017),				
	-	mRNA from Maura				
		Ru .				
H0689	Ovarian Cancer	Ovarian Cancer,				pCMVSport 3.0
		#9806G019				
H0690	Ovarian Cancer, #	Ovarian Cancer,				pCMVSport 3.0
	9702G001	#9702G001	-			
H0691	Normal Ovary,	normal ovary,				pCMVSport 3.
	#9710G208	#9710G208				
H0693	Normal Prostate	Normal Prostate			i	pCMVSport 3.
	#ODQ3958EN	Tissue #				
		ODQ3958EN				
H0694	Prostate gland	Prostate gland,	prostate			pCMVSport 3.
	adenocarcinoma	adenocarcinoma,	gland			Ì
		mod/diff, gleason				
H0695	mononucleocytes from	mononucleocytes				pCMVSport 3.
	patient	from patient at				
70004		Shady Grove Hospit				
N0006	Human Fetal Brain	Human Fetal Brain				<del> </del>
S0001	Brain frontal cortex	Brain frontal cortex	Brain	0.111		Lambda ZAP I
50002	Monocyte activated	Monocyte-activated	blood	Cell Line		Uni-ZAP XR
S0003	Human Osteoclastoma	Osteoclastoma	bone	[	disease	Uni-ZAP XR
50004	Prostate	Prostate BPH	Prostate			Lambda ZAP I
50007	Early Stage Human Brain	Human Fetal Brain		ļ		Uni-ZAP XR
S0010	Human Amygdala	Amygdala				Uni-ZAP XR
S0011	STROMAL -	Osteoclastoma	bone		disease	Uni-ZAP XR
00015	OSTEOCLASTOMA	T21.1				
S0015	Kidney medulla	Kidney medulla	Kidney	l		Uni-ZAP XR
S0016	Kidney Pyramids	Kidney pyramids	Kidney			Uni-ZAP XR
S0021	Whole brain	Whole brain	Brain	0.337		ZAP Express
S0026	Stromal cell TF274	stromal cell	Bone marrow	Cell Line		Uni-ZAP XR
S0027	Smooth muscle, serum treated	Smooth muscle	Pulmanary artery	Cell Line		Uni-ZAP XR
S0028	Smooth muscle,control	Smooth muscle	Pulmanary artery	Cell Line		Uni-ZAP XR
S0031	Spinal cord	Spinal cord	spinal cord			Uni-ZAP XR
S0032	Smooth muscle-ILb	Smooth muscle	Pulmanary	Cell Line		Uni-ZAP XR
	induced		artery	1 1	l	1

S0035	Brain medulla oblongata	Brain medulla oblongata	Brain			Uni-ZAP XR
S0036	Human Substantia Nigra	Human Substantia Nigra				Uni-ZAP XR
S0037	Smooth muscle, IL1b	Smooth muscle	Pulmanary artery	Cell Line		Uni-ZAP XR
S0038	Human Whole Brain #2 - Oligo dT > 1.5Kb	Human Whole Brain #2				ZAP Express
S0040	Adipocytes	Human Adipocytes from Osteoclastoma				Uni-ZAP XR
S0042	Testes	Human Testes				ZAP Express
S0044	Prostate BPH	prostate BPH	Prostate		disease	Uni-ZAP XR
S0045	Endothelial cells-control	Endothelial cell	endothelial cell-lung	Cell Line		Uni-ZAP XR
S0046	Endothelial-induced	Endothelial cell	endothelial cell-lung	Cell Line		Uni-ZAP XR
S0048	Human Hypothalamus, Alzheimer"s	Human Hypothalamus, Alzheimer"s			disease	Uni-ZAP XR
S0049	Human Brain, Striatum	Human Brain, Striatum				Uni-ZAP XR
S0050	Human Frontal Cortex, Schizophrenia	Human Frontal Cortex, Schizophrenia			disease	Uni-ZAP XR
S0051	Human Hypothalmus,Schizophren ia	Human Hypothalamus, Schizophrenia			disease	Uni-ZAP XR
S0052	neutrophils control	human neutrophils	blood	Cell Line		Uni-ZAP XR
S0053	Neutrophils IL-1 and LPS induced	human neutrophil induced	blood	Cell Line		Uni-ZAP XR
S0110	Brain Amygdala Depression		Brain		disease	Uni-ZAP XR
S0114	Anergic T-cell	Anergic T-cell		Cell Line		Uni-ZAP XR
S0116	Bone marrow	Bone marrow	Bone marrow			Uni-ZAP XR
S0126	Osteoblasts	Osteoblasts	Knee	Cell Line		Uni-ZAP XR
S0132	Epithelial-TNFa and INF induced	Airway Epithelial				Uni-ZAP XR
S0134	Apoptotic T-cell	apoptotic cells		Cell Line		Uni-ZAP XR
S0140	eosinophil-IL5 induced	eosinophil	lung	Cell Line		Uni-ZAP XR
S0142	Macrophage-oxLDL	macrophage- oxidized LDL treated	blood	Cell Line		Uni-ZAP XR
S0144	Macrophage (GM-CSF treated)	Macrophage (GM- CSF treated)				Uni-ZAP XR
S0146	prostate-edited	prostate BPH	Prostate			Uni-ZAP XR
S0148	Normal Prostate	Prostate	prostate			Uni-ZAP XR
S0150	LNCAP prostate cell line	LNCAP Cell Line	Prostate	Cell Line		Uni-ZAP XR
S0152	PC3 Prostate cell line	PC3 prostate cell line				Uni-ZAP XR
S0176	Prostate, normal, subtraction I	Prostate	prostate			Uni-ZAP XR
S0182	Human B Cell 8866	Human B- Cell 8866				Uni-ZAP XR
30102	Human B Cen 8800	Tuillan D. Cen 6600				0 242 222 222

		BPH				
S0192	Synovial Fibroblasts (control)	Synovial Fibroblasts				pSport1
S0194	Synovial hypoxia	Synovial Fibroblasts				pSport1
80196	Synovial IL-1/TNF stimulated	Synovial Fibroblasts				pSport1
S0206	Smooth Muscle- HASTE normalized	Smooth muscle	Pulmanary artery	Cell Line		pBluescript
S0210	Messangial cell, frac 2	Messangial cell				pSport1
S0212	Bone Marrow Stromal Cell, untreated	Bone Marrow Stromal Cell,untreated				pSport1
S0214	Human Osteoclastoma, re- excision	Osteoclastoma	bone		disease	Uni-ZAP XR
S0216	Neutrophils IL-1 and LPS induced	human neutrophil induced	blood	Cell Line		Uni-ZAP XR
S0218	Apoptotic T-cell, re- excision	apoptotic cells		Cell Line		Uni-ZAP XR
S0222	H. Frontal cortex,epileptic;re- excision	H. Brain, Frontal Cortex, Epileptic	Brain		disease	Uni-ZAP XR
S0242	Synovial Fibroblasts (II1/TNF), subt	Synovial Fibroblasts				pSport1
S0250	Human Osteoblasts II	Human Osteoblasts	Femur		disease	pCMVSport 2.0
S0260	Spinal Cord, re-excision	Spinal cord	spinal cord			Uni-ZAP XR
S0276	Synovial hypoxia-RSF subtracted	Synovial fobroblasts (rheumatoid)	Synovial tissue			pSport1
S0278	H Macrophage (GM-CSF treated), re-excision	Macrophage (GM- CSF treated)				Uni-ZAP XR
S0280	Human Adipose Tissue, re-excision	Human Adipose Tissue				Uni-ZAP XR
S0282	Brain Frontal Cortex, re- excision	Brain frontal cortex	Brain			Lambda ZAP II
S0294	Larynx tumor	Larynx tumor	Larynx,vocal cord		disease	pSport1
S0298	Bone marrow stroma,treated	Bone marrow stroma,treatedSB	Bone marrow			pSport1
S0300	Frontal lobe,dementia;re- excision	Frontal Lobe dementia/Alzheimer' 's	Brain			Uni-ZAP-XR
S0306	Larynx normal #10 261- 273	Larynx normal				pSport1
S0310	Normal trachea	Normal trachea				pSport1
S0312	Human osteoarthritic;fraction II	Human osteoarthritic cartilage			disease	pSport1
S0314	Human , osteoarthritis;fraction I	Human osteoarthritic cartilage			disease	pSport1
S0316	Human Normal Cartilage,Fraction I	Human Normal Cartilage				pSport1
S0324	Human Brain	Brain	Cerebellum			pSport1
S0328	Palate carcinoma	Palate carcinoma	Uvula		disease	pSport1

S0330	Palate normal	Palate normal	Uvula			pSport1
S0332	Pharynx carcinoma	Pharynx carcinoma	Hypopharynx			pSport1
S0340	Human Osteoarthritic	Human	Пурорнатунх		disease	pSport1
50540	Cartilage Fraction IV	osteoarthritic		1	Giboaso	poporti
	Caratago A raonon A v	cartilage		i		
S0342	Adipocytes;re-excision	Human Adipocytes				Uni-ZAP XR
505.2	Transpoortes, to excision	from Osteoclastoma				Om Din in
S0344	Macrophage-oxLDL; re-	macrophage-	blood	Cell Line		Uni-ZAP XR
	excision	oxidized LDL				
9 11		treated		[ [		
S0346	Human Amygdala;re-	Amygdala				Uni-ZAP XR
)	excision	7.0	]	]		
S0348	Cheek Carcinoma	Cheek Carcinoma			disease	pSport1
S0352	Larynx Carcinoma	Larynx carcinoma			disease	pSport1
S0354	Colon Normal II	Colon Normal	Colon		<u></u>	pSport1
S0356	Colon Carcinoma	Colon Carcinoma	Colon		disease	pSport1
S0358	Colon Normal III	Colon Normal	Colon			pSport1
S0360	Colon Tumor II	Colon Tumor	Colon		disease	pSport1
S0362	Human Gastrocnemius	Gastrocnemius				pSport1
		muscle				
S0364	Human Quadriceps	Quadriceps muscle				pSport1
S0366	Human Soleus	Soleus Muscle				pSport1
S0370	Larynx carcinoma II	Larynx carcinoma			disease	pSport1
S0372	Larynx carcinoma III	Larynx carcinoma			disease	pSport1
S0374	Normal colon	Normal colon				pSport1
\$0376	Colon Tumor	Colon Tumor			disease	pSport1
S0378	Pancreas normal PCA4	Pancreas Normal				pSport1
	No	PCA4 No	<u> </u>	·		
S0380	Pancreas Tumor PCA4 Tu	Pancreas Tumor			disease	pSport1
		PCA4 Tu				
S0384	Tongue carcinoma	Tongue carcinoma			disease	pSport1
S0386	Human Whole Brain, re-	Whole brain	Brain	[ [		ZAP Express
	excision					
S0388	Human	Human		]	disease	Uni-ZAP XR
	Hypothalamus,schizophre	Hypothalamus,				
	nia, re-excision	Schizophrenia				
S0390	Smooth muscle, control;	Smooth muscle	Pulmanary	Cell Line		Uni-ZAP XR
	re-excision		artery			
S0398	Testis; normal	Testis; normal		<u> </u>		pSport1
S0400	Brain; normal	Brain; normal				pSport1
S0404	Rectum normal	Rectum, normal		<u> </u>		pSport1
S0406	Rectum tumour	Rectum tumour				pSport1
S0408	Colon, normal	Colon, normal				pSport1
S0410	Colon, tumour	Colon, tumour				pSport1
S0412	Temporal cortex-	Temporal cortex,	ļ	[	disease	Other
00414	Alzheizmer; subtracted	alzheimer		<b> </b>		-
S0414	Hippocampus, Alzheimer	Hippocampus,				Other
l	Subtracted	Alzheimer	ł			
20419	CUME Call I incidented 5	Subtracted CHME Call Line		<del> </del>		nCMVSnc+20
S0418	CHME Cell Line;treated 5 hrs	CHME Cell Line;				pCMVSport 3.0
S0420		treated CHME Cell line,		<del> </del>		nCport1
00420	CHME Cell	CHIVIE CEII IINE,	l	<u> </u>		pSport1

	Line,untreated	untreatetd			· · · · · · · · · · · · · · · · · · ·	1
S0422	Mo7e Cell Line GM-CSF	Mo7e Cell Line				pCMVSport 3.0
	treated (1ng/ml)	GM-CSF treated				
		(1ng/ml)				
S0424	TF-1 Cell Line GM-CSF	TF-1 Cell Line				pSport1
	Treated	GM-CSF Treated				
S0426	Monocyte activated; re- excision	Monocyte-activated	blood	Cell Line		Uni-ZAP XR
S0428	Neutrophils control; re- excision	human neutrophils	blood	Cell Line		Uni-ZAP XR
S0430	Aryepiglottis Normal	Aryepiglottis Normal				pSport1
S0432	Sinus piniformis Tumour	Sinus piniformis Tumour				pSport1
S0434	Stomach Normal	Stomach Normal		]	disease	pSport1
S0436	Stomach Tumour	Stomach Tumour			disease	pSport1
S0438	Liver Normal Met5No	Liver Normal Met5No				pSport1
S0440	Liver Tumour Met 5 Tu	Liver Tumour			-	pSport1
S0442	Colon Normal	Colon Normal				pSport1
S0444	Colon Tumor	Colon Tumour			disease	pSport1
S0446	Tongue Tumour	Tongue Tumour				pSport1
S0448	Larynx Normal	Larynx Normal				pSport1
S0450	Larynx Tumour	Larynx Tumour	L	<u> </u>		pSport1
S0456	Tongue Normal	Tongue Normal	<del></del>			pSport1
S0458	Thyroid Normal (SDCA2 No)	Thyroid normal				pSport1
S0460	Thyroid Tumour	Thyroid Tumour				pSport1
S0468	Ea.hy.926 cell line	Ea.hy.926 cell line				pSport1
S0474	Human blood platelets	Platelets	Blood platelets			Other
S3010	Human Blastocyst	Human Blastocyst				Other
\$3012	Smooth Muscle Serum Treated, Norm	Smooth muscle	Pulmanary artery	Cell Line		pBluescript
\$3014	Smooth muscle, serum induced,re-exc	Smooth muscle	Pulmanary artery	Cell Line	<del></del>	pBluescript
S6014	H. hypothalamus, frac A	Hypothalamus	Brain			ZAP Express
S6016	H. Frontal Cortex, Epileptic	H. Brain, Frontal Cortex, Epileptic	Brain		disease	Uni-ZAP XR
S6022	H. Adipose Tissue	Human Adipose Tissue				Uni-ZAP XR
S6024	Alzheimers, spongy change	Alzheimer"s/Spongy change	Brain		disease	Uni-ZAP XR
S6026	Frontal Lobe, Dementia	Frontal Lobe dementia/Alzheimer' 's	Brain			Uni-ZAP XR
S6028	Human Manic Depression Tissue	Human Manic depression tissue	Brain		disease	Uni-ZAP XR
T0002	Activated T-cells	Activated T-Cell, PBL fraction	Blood	Cell Line		pBluescript SK-
T0003	Human Fetal Lung	Human Fetal Lung				pBluescript SK-
T0006	Human Pineal Gland	Human Pinneal				pBluescript SK-

		Gland				
T0008	Colorectal Tumor	Colorectal Tumor			disease	pBluescript SK-
T0010	Human Infant Brain	Human Infant Brain				Other
T0023	Human Pancreatic	Human Pancreatic			disease	pBluescript SK-
	Carcinoma	Carcinoma				
T0039	HSA 172 Cells	Human HSA172 cell				pBluescript SK-
		line		<u> </u>		
T0040	HSC172 cells	SA172 Cells	<u></u>	ļ <u>.</u>		pBluescript SK-
T0041	Jurkat T-cell G1 phase	Jurkat T-cell				pBluescript SK-
T0042	Jurkat T-Cell, S phase	Jurkat T-Cell Line		<del> </del>		pBluescript SK-
T0048	Human Aortic	Human Aortic		1		pBluescript SK-
	Endothelium	Endothilium		ļļ.		_
T0049	Aorta endothelial cells +	Aorta endothelial				pBluescript SK-
*****	TNF-a	cells				
T0060	Human White Adipose	Human White Fat		<del> </del>		pBluescript SK-
T0067	Human Thyroid	Human Thyroid		<b> </b>		pBluescript SK-
T0068	Normal Ovary,	Normal Ovary,				pBluescript SK-
T0069	Premenopausal	Premenopausal				-Dii CIZ
10009	Human Uterus, normal	Human Uterus, normal		1		pBluescript SK-
T0071	Human Bone Marrow	Human Bone		<del> </del>		pBluescript SK-
10071	Tidinali Dolle Mallow	Marrow		1.		photoescript six-
T0074	Human Adult Retina	Human Adult Retina				pBluescriptISK-
T0078	Human Liver, normal	Human Liver,				pBluescript SK-
100,0	adult	normal Adult		1		p p p r a c s c r r r r r r r r r r r r r r r r r
T0082	Human Adult Retina	Human Adult Retina				pBluescript SK-
T0109	Human (HCC) cell line					pBluescript SK-
	liver (mouse) metastasis,					
	remake					
T0110	Human colon carcinoma					pBluescript SK-
	(HCC) cell line, remake			<u> </u>		
T0114	Human (Caco-2) cell line,	]				pBluescript SK-
	adenocarcinoma, colon,			1		
	remake			-		
T0115	Human Colon Carcinoma	l		1		pBluescript SK-
	(HCC) cell line			ļ		<u> </u>
L0005	Clontech human aorta					i ·
T 0001	polyA+ mRNA (#6572)					
L0021	Human adult (K.Okubo)					
L0040	Human colon mucosa				<del></del>	
L0055	Human promyelocyte			<del> </del>		
L0060	Human thymus NSTH II			<del> </del>		<u> </u>
L0065 L0105	Liver HepG2 cell line.  Human aorta polyA+	norte		<del>                                     </del>		
T0100	(TFujiwara)	aorta				
L0142	Human placenta cDNA	placenta		<del>                                     </del>		
101-72	(TFujiwara)	Pracenta				1
L0146	Human fovea cDNA	retinal fovea				
L0151	Human testis (C. De	testis	·			
	Smet)			1		İ
L0157	Human fetal brain		brain			
	(TFujiwara)	]		]		

L0163	Human heart cDNA		heart		 1
20105	(YNakamura)		nou.t		
L0174	AP20 melanoma mRNA			AP20	
				melanom	
				a	
L0185	Human immortalized			HS74 and	
	fibroblasts (H.L.Ozer)			its SV40-	
		Ì		transform	
				ed	
Y 00.51				sublines	 D. 1.740
L0351	Infant brain, Bento Soares	Į			BA, M13-
L0352	Normalized infant brain,				 derived
L0332	Bento Soares				BA, M13- derived
L0356	S, Human foetal Adrenals				Bluescript
10330	tissue	ĺ		1	Didescript
L0361	Stratagene ovary		ovary		 Bluescript SK
	(#937217)	}	,		
L0362	Stratagene ovarian cancer		4		 Bluescript SK-
	(#937219)				 
L0363	NCI_CGAP_GC2	germ cell tumor			Bluescript SK-
L0364	NCI_CGAP_GC5	germ cell tumor			 Bluescript SK-
L0366	Stratagene schizo brain	schizophrenic brain		1	Bluescript SK-
	S11	S-11 frontal lobe			
L0367	NCI_CGAP_Sch1	Schwannoma tumor			 Bluescript SK-
L0368	NCI_CGAP_SS1	synovial sarcoma			 Bluescript SK-
L0369	NCI_CGAP_AA1	adrenal adenoma	adrenal gland		 Bluescript SK-
L0370	Johnston frontal cortex	pooled frontal lobe	brain		 Bluescript SK-
L0372	NCI_CGAP_Co12	colon tumor	colon		 Bluescript SK-
L0373	NCI_CGAP_Co11	tumor	colon		 Bluescript SK-
L0374	NCI_CGAP_Co2	tumor	colon	<b> </b>	 Bluescript SK-
L0375	NCI_CGAP_Kid6	kidney tumor	kidney .		 Bluescript SK-
L0376	NCI_CGAP_Lar1	larynx	larynx		 Bluescript SK-
L0378	NCI_CGAP_Lu1_	lung tumor	lung	<u> </u>	 Bluescript SK-
L0381	NCI_CGAP_HN4	squamous cell	pharynx		Bluescript SK-
7.0000	NOT COAD DOS	carcinoma		<del>[</del>	 Di corr
L0382	NCI_CGAP_Pr25	epithelium (cell line)	prostate		 Bluescript SK-
L0383	· NCI_CGAP_Pr24	invasive tumor (cell	prostate		Bluescript SK-
L0384	NCI_CGAP_Pr23	line) prostate tumor	prostate		 Bluescript SK-
L0386	NCI_CGAP_HN3	squamous cell	tongue		 Bluescript SK-
1 20300	HOLCOM THIS	carcinoma from base	longue	]	Dinescribe 217-
		of tongue		]	
L0387	NCI_CGAP_GCB0	germinal center B-	tonsil		 Bluescript SK-
	- · · · - · · ·	cells			
L0388	NCI_CGAP_HN6	normal gingiva (cell			 Bluescript SK-
		line from			1
		immortalized kerati			 
L0393	B, Human Liver tissue				gt11
L0415	b4HB3MA Cot8-HAP-Ft				 Lafmid BA
L0418	b4HB3MA-Cot109+10-	_			Lafmid BA
	Bio			<u>[l</u>	

T 0425	Y Court of TINE		1		1.6.154
L0435	Infant brain, LLNL array of Dr. M. Soares 1NIB				lafmid BA
L0438	normalized infant brain cDNA	total brain	brain		lafmid BA
L0439	Soares infant brain 1NIB		whole brain		Lafmid BA
L0443	b4HB3MK		WHOIC OILLII		Lafmid BK
L0455	Human retina cDNA	retina	eye		lambda gt10
20.50	randomly primed				Talmoda gero
ļ	sublibrary				
L0462	WATM1				lambda gt11
L0465	TEST1, Human adult				lambda nm1149
	Testis tissue		1		1
L0468	HE6W				lambda zap
L0471	Human fetal heart,				Lambda ZAP
	Lambda ZAP Express				Express
L0475	KG1-a Lambda Zap			KG1-a	Lambda Zap
	Express cDNA library		[	ĺ	Express
					(Stratagene)
L0480	Stratagene cat#937212	j			Lambda ZAP,
	(1992)		1		pBluescript
					SK(-)
L0483	Human pancreatic islet				Lambda ZAPII
L0485	STRATAGENE Human	skeletal muscle	leg muscle	ĺ	Lambda ZAPII
	skeletal muscle cDNA			ļ	
	library, cat. #936215.	<u> </u>			
L0493	NCI_CGAP_Ov26	papillary serous carcinoma	ovary		pAMP1
L0499	NCI_CGAP_HSC2	stem cell 34+/38+	bone marrow		pAMP1
L0509	NCI_CGAP_Lu26	invasive	lung	1	pAMP1
		adenocarcinoma			
L0513	NCI_CGAP_Ov37	early stage papillary	ovary		pAMP1
		serous carcinoma			
L0517	NCI_CGAP_Pr1	ļ			pAMP10
L0518	NCI_CGAP_Pr2	<del> </del>			pAMP10
L0519	NCI_CGAP_Pr3				pAMP10
L0520	NCI_CGAP_Alv1	alveolar	.		pAMP10
ļ		rhabdomyosarcoma			
L0521	NCI_CGAP_Ew1	Ewing's sarcoma			pAMP10
L0522	NCI_CGAP_Kid1	kidney			pAMP10
L0523	NCI_CGAP_Lip2	liposarcoma			pAMP10
L0524	NCI_CGAP_Li1	liver			pAMP10
L0525	NCI_CGAP_Li2	liver			pAMP10
L0526	NCI_CGAP_Pr12	metastatic prostate	}	}	pAMP10
- T 0505	NOT COAR O	bone lesion		<del></del>	11.554
L0527	NCI_CGAP_Ov2	ovary			pAMP10
L0528	NCI_CGAP_Pr5	prostate			pAMP10
L0529	NCI_CGAP_Pr6	prostate			pAMP10
L0530	NCI_CGAP_Pr8	prostate			pAMP10
L0532	NCI_CGAP_Thy1	thyroid			pAMP10
L0533	NCI_CGAP_HSC1	stem cells	bone marrow		pAMP10
L0534	Chromosome 7 Fetal	brain	brain		pAMP10
L	Brain cDNA Library	<u> </u>	L	,,	

L0539	Chromosome 7 Placental		placenta	T	pAMP10
LOJJJ	cDNA Library	[	placenta		pawa 10
L0540	NCI_CGAP_Pr10	invasive prostate tumor	prostate		pAMP10
L0541	NCI_CGAP_Pr7	low-grade prostatic	prostate		pAMP10
L0543	NCI_CGAP_Pr9	normal prostatic epithelial cells	prostate		pAMP10
L0545	NCI_CGAP_Pr4.1	prostatic intraepithelial neoplasia - high grade	prostate		pAMP10
L0546	NCI_CGAP_Pr18	stroma	prostate		pAMP10
L0550	NCI_CGAP_HN9	normal squamous epithelium from retromolar trigone			pAMP10
L0551	NCI_CGAP_HN7	normal squamous epithelium, floor of mouth			pAMP10
L0554	NCI_CGAP_Li8		liver		pAMP10
L0562	Chromosome 7 HeLa cDNA Library			HeLa cell line;	pAMP10
L0564	Jia bone marrow stroma	bone marrow stroma			pBluescript
L0565	Normal Human Trabecular Bone Cells	Bone	Hip		pBluescript
L0581	Stratagene liver (#937224)		liver		pBluescript SK
L0583	Stratagene cDNA library Human fibroblast, cat#937212				pBluescript SK(+)
L0584	Stratagene cDNA library Human heart, cat#936208				pBluescript SK(+)
L0586	HTCDL1				pBluescript SK(-)
L0587	Stratagene colon HT29 (#937221)				pBluescript SK-
L0588	Stratagene endothelial cell 937223				pBluescript SK-
L0589	Stratagene fetal retina 937202				pBluescript SK-
L0591	Stratagene HeLa cell s3 937216				pBluescript SK-
L0592	Stratagene hNT neuron (#937233)				pBluescript SK-
L0593	Stratagene neuroepithelium (#937231)				pBluescript SK-
L0594	Stratagene neuroepithelium NT2RAMI 937234				pBluescript SK-
L0595	Stratagene NT2 neuronal precursor 937230	neuroepithelial cells	brain		pBluescript SK-
L0596	Stratagene colon		colon		pBluescript SK-

	(#937204)				
L0598	Morton Fetal Cochlea	cochlea	ear		pBluescript SK-
L0599	Stratagene lung (#937210)		lung		pBluescript SK-
L0600	Weizmann Olfactory Epithelium	olfactory epithelium	nose		pBluescript SK-
L0601	Stratagene pancreas (#937208)		pancreas		pBluescript SK-
L0602	Pancreatic Islet	pancreatic islet	pancreas		pBluescript SK-
L0603	Stratagene placenta (#937225)		placenta		pBluescript SK-
L0604	Stratagene muscle 937209	muscle	skeletal muscle		pBluescript SK-
L0605	Stratagene fetal spleen (#937205)	fetal spleen	spleen		pBluescript SK-
L0606	NCI_CGAP_Lym5	follicular lymphoma	lymph node		pBluescript SK-
L0608	Stratagene lung carcinoma 937218	lung carcinoma	lung	NCI-H69	pBluescript SK-
L0612	Schiller oligodendroglioma	oligodendroglioma	brain		pBluescript SK- (Stratagene)
L0615	22 week old human fetal liver cDNA library				pBluescriptII SK(-)
L0617	Chromosome 22 exon				pBluescriptIIKS
L0622	HM1				pcDNAII (Invitrogen)
L0623	НМ3	pectoral muscle (after mastectomy)	•		pcDNAII (Invitrogen)
L0626	NCI_CGAP_GC1	bulk germ cell seminoma			pCMV-SPORT
L0629	NCI_CGAP_Mel3	metastatic melanoma to bowel	bowel (skin primary)		pCMV-SPORT
L0631	NCI_CGAP_Br7	,	breast		pCMV-SPORT4
L0632	NCI_CGAP_Li5	hepatic adenoma	liver		pCMV-SPORT
L0634	NCI_CGAP_Ov8	serous adenocarcinoma	ovary		pCMV-SPORT
L0636	NCI_CGAP_Pit1	four pooled pituitary adenomas	brain		pCMV-SPORT
L0637	NCI_CGAP_Brn53	three pooled meningiomas	brain		pCMV-SPORT
L0638	NCI_CGAP_Brn35	tumor, 5 pooled (see description)	brain		pCMV-SPORT
L0639	NCI_CGAP_Brn52	tumor, 5 pooled (see description)	brain		pCMV-SPORT
L0640	NCI_CGAP_Br18	four pooled high- grade tumors, including two prima	breast		pCMV-SPORT
L0641	NCI_CGAP_Co17	juvenile granulosa tumor	colon		pCMV-SPORT
L0642	NCI_CGAP_Co18	moderately differentiated adenocarcinoma	colon		pCMV-SPORT
L0643	NCI_CGAP_Co19	moderately differentiated	colon		pCMV-SPORT

	····				·
<del></del>	<u> </u>	adenocarcinoma			
L0644	NCI_CGAP_Co20	moderately	colon		pCMV-SPORT6
		differentiated			
	ļ	adenocarcinoma			
L0645	NCI_CGAP_Co21	moderately	colon	ļ	pCMV-SPORT6
		differentiated			
		adenocarcinoma			
L0646	NCI_CGAP_Co14	moderately-	colon	ł	pCMV-SPORT6
		differentiated		1	
		adenocarcinoma		}	1
L0647	NCI_CGAP_Sar4	five pooled	connective		pCMV-SPORT6
		sarcomas, including	tissue		,
		myxoid liposarcoma			
L0648	NCI_CGAP_Eso2	squamous cell	esophagus		pCMV-SPORT6
		carcinoma			F
L0649	NCI_CGAP_GU1	2 pooled high-grade	genitourinary		pCMV-SPORT6
-200.5		transitional cell	tract		point brokers
		tumors			
L0650	NCI_CGAP_Kid13	2 pooled Wilms"	kidney		pCMV-SPORT6
20020	1101_00111 _111115	tumors, one primary	l Ridney		pelvi v-bi okto
		and one metast			
L0651	NCI_CGAP_Kid8	renal cell tumor	kidney		pCMV-SPORT6
L0653	NCI_CGAP_Lu28	two pooled	lung		pCMV-SPORT6
1.0033	NCI_COAI_Luzo	squamous cell	lung		pcwv-srokio
	,	carcinomas			İ
L0655	NCI CGAD I vizz 12		1001010		CN (IV ODODTIC
L0033	NCI_CGAP_Lym12	lymphoma,	lymph node		pCMV-SPORT6
		follicular mixed			
1.0656	NOI COAD O-20	small and large cell			C) GI CDODEC
L0656	NCI_CGAP_Ov38	normal epithelium	ovary		pCMV-SPORT6
L0657	NCI_CGAP_Ov23	tumor, 5 pooled (see	ovary	- 1	pCMV-SPORT6
7.0650	YOU GOLD O OF	description)			
L0658	NCI_CGAP_Ov35	tumor, 5 pooled (see	ovary	Į.	pCMV-SPORT6
		description)			
L0659	NCI_CGAP_Pan1	adenocarcinoma	pancreas		pCMV-SPORT6
L0661	NCI_CGAP_Mel15	malignant	skin		pCMV-SPORT6
		melanoma,			
	ľ	metastatic to lymph			1
-		node			
L0662	NCI_CGAP_Gas4	poorly differentiated	stomach		pCMV-SPORT6
		adenocarcinoma			
		with signet r			
L0663	NCI_CGAP_Ut2	moderately-	uterus	•	pCMV-SPORT6
		differentiated			
		endometrial			
		adenocarcino			
L0664	NCI_CGAP_Ut3	poorly-differentiated	uterus		pCMV-SPORT6
		endometrial			
		adenocarcinoma,			
L0665	NCI_CGAP_Ut4	serous papillary	uterus		pCMV-SPORT6
		carcinoma, high			
		grade, 2 pooled t			
			<del></del>		<del></del>
L0666	NCI_CGAP_Ut1	well-differentiated	uterus		pCMV-SPORT6

		adenocarcinoma, 7			
L0667	NCI_CGAP_CML1	myeloid cells, 18	whole blood		pCMV-SPORT6
		pooled CML cases,			
		BCR/ABL rearra			
L0683	Stanley Frontal NS pool 2	frontal lobe (see	brain		pCR2.1-TOPO
		description)			(Invitrogen)
L0686	Stanley Frontal SN pool 2	frontal lobe (see	brain		pCR2.1-TOPO
		description)			(Invitrogen)
L0697	Testis 1				PGEM 5zf(+)
L0698	Testis 2				PGEM 5zf(+)
L0717	Gessler Wilms tumor				pSPORT1
L0719	human embryo cDNA library	Whole embryo			pSPORT1
L0731	Soares_pregnant_uterus_ NbHPU		uterus		pT7T3-Pac
L0738	Human colorectal cancer				pT7T3D
L0740	Soares melanocyte	melanocyte			pT7T3D
	2NbHM		J		(Pharmacia)
					with a modified
					polylinker
L0741	Soares adult brain		brain		pT7T3D
	N2b4HB55Y				(Pharmacia)
					with a modified
					polylinker
L0742	Soares adult brain		brain		pT7T3D
	N2b5HB55Y		1		(Pharmacia)
					with a modified
					polylinker
L0743	Soares breast 2NbHBst		breast		pT7T3D
					(Pharmacia)
			i	1 1	with a modified
	<u> </u>				polylinker
L0744	Soares breast 3NbHBst		breast		pT7T3D
	ı		ļ		(Pharmacia)
					with a modified
T 05745	C NOLATE				polylinker
L0745	Soares retina N2b4HR	retina	eye		pT7T3D
					(Pharmacia) with a modified
			•		
L0746	Soares retina N2b5HR	retina	AVIO	<del> </del>	polylinker pT7T3D
L0140	Source letting INZOSTIK	1 euna	eye		(Pharmacia)
					with a modified
					polylinker
L0747	Soares_fetal_heart_NbHH		heart		pT7T3D
= 10,747	19W		, mair		(Pharmacia)
					with a modified
					polylinker
L0748	Soares fetal liver spleen		Liver and		pT7T3D
	1NFLS		Spleen		(Pharmacia)
					with a modified
			}	1	polylinker
L0749	Soares_fetal_liver_spleen		Liver and		pT7T3D

	_1NFLS_S1		Spleen		(Pharmacia)
					with a modified
			]	1	polylinker
L0750	Soares_fetal_lung_NbHL1		lung		 pT7T3D
	9W		1	}	(Pharmacia)
					with a modified
	}		1		polylinker
L0751	Soares ovary tumor	ovarian tumor	ovary		pT7T3D
	NЬНОТ	·		ĺ	(Pharmacia)
			}	1	with a modified
					polylinker
L0752	Soares_parathyroid_tumor	parathyroid tumor	parathyroid		pT7T3D
	_NbHPA	1 . 3	gland		(Pharmacia)
				ł	with a modified
					polylinker
L0753	Soares_pineal_gland_N3H		pineal gland		pT7T3D
	PG		1 0	1	(Pharmacia)
			ĺ	Ĭ	with a modified
			ļ	1	polylinker
L0754	Soares placenta Nb2HP		placenta		pT7T3D
	-		ļ [*]		(Pharmacia)
			7		with a modified
			l	ł	polylinker
L0755	Soares_placenta_8to9wee		placenta		pT7T3D
	ks_2NbHP8to9W		-	1	(Pharmacia)
			i	ļ	with a modified
				<u> </u>	polylinker
L0756	Soares_multiple_sclerosis	multiple sclerosis			pT7T3D
	_2NbHMSP	lesions		1	(Pharmacia)
					with a modified
			}	}	polylinker
					V_TYPE
L0757	Soares_senescent_fibrobla	senescent fibroblast		1	pT7T3D
	sts_NbHSF				(Pharmacia)
					with a modified
					polylinker
					V_TYPE
L0758	Soares_testis_NHT		]	1	pT7T3D-Pac
	•				(Pharmacia)
			}	l .	with a modified
					 polylinker
L0759	Soares_total_fetus_Nb2H				pT7T3D-Pac
	F8_9w				(Pharmacia)
			[	[	with a modified
					 polylinker
L0760	Barstead aorta HPLRB3	aorta			pT7T3D-Pac
					(Pharmacia)
					with a modified
•				<b>_</b>	 polylinker
L0761	NCI_CGAP_CLL1	B-cell, chronic	ļ		pT7T3D-Pac
	•	lymphotic leukemia	l		(Pharmacia)
					with a modified
*				<u> </u>	 polylinker
L0762	NCI_CGAP_Br1.1	breast	ł	<u> </u>	pT7T3D-Pac

		<del></del>		T	· · · · · · · · · · · · · · · · · · ·	
e ac						(Pharmacia)
						with a modified
					ļ <u>.</u>	polylinker
L0763	NCI_CGAP_Br2	breast				pT7T3D-Pac
Į				ļ		(Pharmacia)
						with a modified
						polylinker
L0764	NCI_CGAP_Co3	colon		ļ		pT7T3D-Pac
						(Pharmacia)
	P			ļ		with a modified
						polylinker
L0765	NCI_CGAP_Co4	colon				pT7T3D-Pac
				J		(Pharmacia)
				ļ		with a modified
				]		polylinker
L0766	NCI_CGAP_GCB1	germinal center B		Ę		pT7T3D-Pac
		ceil		1		(Pharmacia)
		•		ļ		with a modified
				<u> </u>		polylinker
L0767	NCI_CGAP_GC3	pooled germ cell				pT7T3D-Pac
) .		tumors			•	(Pharmacia)
	1		•	1		with a modified
					ļ	polylinker
L0768	NCI_CGAP_GC4	pooled germ cell		1		pT7T3D-Pac
		tumors		Į		(Pharmacia)
				1		with a modified
			***		<u> </u>	polylinker
L0769	NCI_CGAP_Brn25	anaplastic	brain			pT7T3D-Pac
j .		oligodendroglioma		ļ		(Pharmacia)
						with a modified
					<u> </u>	polylinker
L0770	NCI_CGAP_Brn23	glioblastoma	brain		1	pT7T3D-Pac
		(pooled)		1		(Pharmacia)
		}		ļ		with a modified
						polylinker
L0771	NCI_CGAP_Co8	adenocarcinoma	colon	ļ		pT7T3D-Pac
						(Pharmacia)
				ļ		with a modified
				]		polylinker
L0772	NCI_CGAP_Co10	colon tumor RER+	colon	<u> </u>		pT7T3D-Pac
[				i		(Pharmacia)
					ŀ	with a modified
			·		ļ <u>.</u>	polylinker
L0773	NCI_CGAP_Co9	colon tumor RER+	colon	1		pT7T3D-Pac
					1	(Pharmacia)
				1		with a modified
<u> </u>				ļ	<u> </u>	polylinker
L0774	NCI_CGAP_Kid3		kidney			pT7T3D-Pac
					1	(Pharmacia)
				]		with a modified
						polylinker
L0775	NCI_CGAP_Kid5	2 pooled tumors	kidney	}	}	pT7T3D-Pac
		(clear cell type)				(Pharmacia)
L					<u></u>	with a modified

		T				polylinker
L0776	NCI_CGAP_Lu5	carcinoid	lung			pT7T3D-Pac
Lorro	Trei_ed/ii _bas	Carcinoid	lung	] ]		(Pharmacia)
						with a modified
						polylinker
L0777	Soares_NhHMPu_S1	Pooled human	mixed (see			pT7T3D-Pac
LOTT	Soares_IVIIIIVII u_S1	melanocyte, fetal	below)			(Pharmacia)
]	•	heart, and pregnant	below)	]		with a modified
		neart, and pregnant				polylinker
L0779	Secret NEL T CPC S1		pooled			pT7T3D-Pac
LUTTS	Soares_NFL_T_GBC_S1		poored	1		(Pharmacia)
		ĺ				with a modified
J		]	j	]		polylinker
1.0790	Soores NSE ES OW OT		pooled	<del> </del>		
L0780	Soares_NSF_F8_9W_OT		pooled			pT7T3D-Pac
	_PA_P_S1					(Pharmacia) with a modified
						polylinker
1.0792	NCL CCAD D-01	named mastata				
L0782	NCI_CGAP_Pr21	normal prostate	prostate			pT7T3D-Pac
ļ	Í		1			(Pharmacia) with a modified
				1		
1.0702	NCL CCAD D-00	11				polylinker
L0783	NCI_CGAP_Pr22	normal prostate	prostate			pT7T3D-Pac
						(Pharmacia) with a modified
Ï						polylinker
L0784	NCI_CGAP_Lei2	10:000000000000000000000000000000000000	soft tissue	<del> </del>		
LU/04	NCI_COAF_Lei2	leiomyosarcoma	Soft ussue			pT7T3D-Pac (Pharmacia)
						with a modified
						polylinker
L0785	Barstead spleen HPLRB2		spleen	<del>  </del>		pT7T3D-Pac
10763	Darstead spicen in LKD2		spicen			(Pharmacia)
	{			1		with a modified
	'					polylinker
L0786	Soares_NbHFB		whole brain	<del>                                     </del>		pT7T3D-Pac
L0700	Sourcs_IVOIT D		Whole ofain			(Pharmacia)
						with a modified
	1	ł	}	1	:	polylinker
L0787	NCI_CGAP_Sub1					pT7T3D-Pac
10,0,	Trei_eerii _eaer		]	j J		(Pharmacia)
						with a modified
İ			Ì	1		polylinker
L0788	NCI_CGAP_Sub2					pT7T3D-Pac
20,00	1.31_00111_0002	1	1	1		(Pharmacia)
						with a modified
		1				polylinker
L0789	NCI_CGAP_Sub3		[			pT7T3D-Pac
20.05			1			(Pharmacia)
			Į.			with a modified
						polylinker
L0790	NCI_CGAP_Sub4			<del> </del>		pT7T3D-Pac
20750	1.01_00/11_0007			1		(Pharmacia)
						with a modified
			1	1 1		polylinker
L	L	L	l	اـــــــــــــــــــــــــــــــــــــ		Porjunicon

Y 0701 VIG	2017 0 15			T	1	-T7T2D D
L0791 NC	[_CGAP_Sub5			l	{	pT7T3D-Pac
					i	(Pharmacia)
						with a modified
				<u> </u>		polylinker
L0792 NC	L_CGAP_Sub6					pT7T3D-Pac
						(Pharmacia)
1						with a modified
						polylinker
L0794 NC	I_CGAP_GC6	pooled germ cell				pT7T3D-Pac
		tumors		İ		(Pharmacia)
ļ						with a modified
						polylinker
L0796 NC	I_CGAP_Brn50	medulloblastoma	brain			pT7T3D-Pac
						(Pharmacia)
	,			1		with a modified
						polylinker
L0800 NC	I_CGAP_Co16	colon tumor, RER+	colon			pT7T3D-Pac
Locott		oolon valler, xuller				(Pharmacia)
						with a modified
						polylinker
L0803 NC	I_CGAP_Kid11		kidney			pT7T3D-Pac
LUGUS   INC	I_CGWL_IXIGIT		Ridiicy			(Pharmacia)
İ				Į		with a modified
1		!				polylinker
7 0004 NG	I CCAD IZ: 110	01- 1	1-1-1	<del></del>	<del> </del>	pT7T3D-Pac
L0804 NC	I_CGAP_Kid12	2 pooled tumors	kidney			(Pharmacia)
		(clear cell type)				with a modified
7.000				-		polylinker
L0805 NC	I_CGAP_Lu24	carcinoid	lung			pT7T3D-Pac
						(Pharmacia)
						with a modified
			<u> </u>		·	polylinker
L0806 NC	I_CGAP_Lu19	squamous cell	lung			pT7T3D-Pac
		carcinoma, poorly		1		(Pharmacia)
		differentiated (4		ļ		with a modified
						polylinker
L0807 NC	I_CGAP_Ov18	fibrotheoma	ovary		1	pT7T3D-Pac
						(Pharmacia)
						with a modified
	(3.)			ļ		polylinker
L0808 Bas	rstead prostate BPH		prostate			pT7T3D-Pac
HP	LRB4 1		ļ		1	(Pharmacia)
			i			with a modified
						polylinker
L0809 NC	L_CGAP_Pr28		prostate			pT7T3D-Pac
		Į				(Pharmacia)
			ļ			with a modified
						polylinker
T 2250 TT						
L2250 Hu	man cerebral cortex	cerebral cortex				

## TABLE 5

OMIM	Description
Reference	
103850	Aldolase A deficiency
106165	Hypertension, essential, 145500
107470	Atypical mycobacterial infection, familial disseminated, 209950
107470	BCG infection, generalized familial
107470	Tuberculosis, susceptibility to
107777	Diabetes insipidus, nephrogenic, autosomal recessive, 222000
107970	Arrhythmogenic right ventricular dysplasia-1
108730	Brody myopathy, 601003
114835	Monocyte carboxyesterase deficiency
115650	Cataract, anterior polar-1
116800	Cataract, Marner type
117700	[Hypoceruloplasminemia, hereditary]
117700	Hemosiderosis, systemic, due to aceruloplasminemia
120110	Metaphyseal chondrodysplasia, Schmid type
121014	Heterotaxia, visceroatrial, autosomal recessive
123270	[Creatine kinase, brain type, ectopic expression of]
123940	White sponge nevus, 193900
126451	Schizophrenia, susceptibility to
126650	Chloride diarrhea, congenital, Finnish type, 214700
126650	Colon cancer
139350	Epidermolytic hyperkeratosis, 113800
139350	Keratoderma, palmoplantar, nonepidermolytic
140100	[Anhaptoglobinemia]
140100	[Hypohaptogloginemia]
147781	Atopy, susceptibility to
148040	Epidermolysis bullosa simplex, Koebner, Dowling-Meara, and Weber-
	Cockayne types, 131900, 131760, 131800
148041	Pachyonychia congenita, Jadassohn-Lewandowsky type, 167200
148043	Meesmann corneal dystrophy, 122100
148070	Liver disease, susceptibility to, from hepatotoxins or viruses
150210	Lactoferrin-deficient neutrophils, 245480
154276	Malignant hyperthermia susceptibility 3
164200	Oculodentodigital dysplasia
164200	Syndactyly, type III, 186100
169600	Hailey-Hailey disease
172471	Glycogenosis, hepatic, autosomal
173360	Thrombophilia due to excessive plasminogen activator inhibitor
173360	Hemorrhagic diathesis due to PAI1 deficiency
180380	Night blindness, congenital stationery, rhodopsin-related
180380	Retinitis pigmentosa, autosomal recessive
180380	Retinitis pigmentosa-4, autosomal dominant
182600	Spastic paraplegia-3A
186580	Arthrocutaneouveal granulomatosis

190000	Atransferrinemia
192090	Ovarian carcinoma
192090	Breast cancer, lobular
192090	Endometrial carcinoma
192090	Gastric cancer, familial, 137215
203500	Alkaptonuria
231550	Achalasia-addisonianism-alacrimia syndrome
232050	Propionicacidemia, type II or pccB type
245200	Krabbe disease
245900	Norum disease
245900	Fish-eye disease
250100	Metachromatic leukodystrophy
250800	Methemoglobinemia, type I
250800	Methemoglobinemia, type II
251600	Microphthalmia, autosomal recessive
261640	Phenylketonuria due to PTS deficiency
264800	Pseudoxanthoma elasticum
266600	Inflammatory bowel disease-1
270100	Situs inversus viscerum
276600	Tyrosinemia, type II
276900	Usher syndrome, type 1A
276902	Usher syndrome, type 3
278760	Xeroderma pigmentosum, group F
300047	Mental retardation, X-linked 20
300062	Mental retardation, X-linked 14
300071	Night blindness, congenital stationary, type 2
300110	Night blindness, congenital stationary, X-linked incomplete, 300071
300600	Ocular albinism, Forsius-Eriksson type
301000	Thrombocytopenia, X-linked, 313900
301000	Wiskott-Aldrich syndrome
301830	Arthrogryposis, X-linked (spinal muscular atrophy, infantile, X-linked)
309470	Mental retardation, X-linked, syndromic-3, with spastic diplegia
309500	Renpenning syndrome-1
309610	Mental retardation, X-linked, syndromic-2, with dysmorphism and
	cerebral atrophy
309850	Brunner syndrome
310500	Night blindness, congenital stationary, type 1
310600	Norrie disease
310600	Exudative vitreoretinopathy, X-linked, 305390
311050	Optic atrophy, X-linked
312060 .	Properdin deficiency, X-linked
600194	Ichthyosis bullosa of Siemens, 146800
600223	Spinocerebellar ataxia-4
600231	Palmoplantar keratoderma, Bothnia type
600536	Myopathy, congenital
600760	Pseudohypoaldosteronism, type I, 264350
<del></del>	1

600760	Liddle syndrome, 177200
600761	Pseudohypoaldosteronism, type I, 264350
600761	Liddle syndrome, 177200
600808	Enuresis, nocturnal, 2
600882	Charcot-Marie-Tooth neuropathy-2B
600956	Persistent Mullerian duct syndrome, type II, 261550
601199	Neonatal hyperparathyroidism, 239200
601199	Hypocalcemia, autosomal dominant, 601198
601199	Hypocalciuric hypercalcemia, type I, 145980
601284	Hereditary hemorrhagic telangiectasia-2, 600376
601316	Deafness, autosomal dominant 10
601471	Moebius syndrome-2
601666	Insulin-dependent diabetes mellitus-15
601682	Glaucoma 1C, primary open angle
601757	Rhizomelic chondrodysplasia punctata, type 1, 215100
601769	Osteoporosis, involutional
601769	Rickets, vitamin D-resistant, 277440
601928	Monilethrix, 158000
602066	Convulsions, infantile and paroxysmal choreoathetosis
602091	Marfan syndrome, atypical
602116	Glioma
602136	Refsum disease, infantile, 266510
602136	Zellweger syndrome-1, 214100
602136	Adrenoleukodystrophy, neonatal, 202370
602153	Monilethrix, 158000
602447	Coronary artery disease, susceptibility to
602574	Deafness, autosomal dominant 12, 601842
602574	Deafness, autosomal dominant 8, 601543
602772	Retinitis pitmentosa-24

## Polynucleotide and Polypeptide Variants

[82] The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X or the complementary strand thereto, nucleotide sequences encoding the polypeptide of SEQ ID NO:Y, the nucleotide sequence of SEQ ID NO:X encoding the polypeptide sequence as defined in column 7 of Table 1A, nucleotide sequences encoding the polypeptide as defined in column 7 of Table 1A, the nucleotide sequence as defined in columns 8 and 9 of Table 2, nucleotide sequences encoding the polypeptide encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2, the nucleotide sequence as defined in column 6 of Table 1B, nucleotide sequences encoding the polypeptide encoded by the nucleotide sequence as defined in column 6 of Table 1B, the

cDNA sequence contained in Clone ID NO:Z, and/or nucleotide sequences encoding the polypeptide encoded by the cDNA sequence contained in Clone ID NO:Z.

[83] The present invention also encompasses variants of the polypeptide sequence disclosed in SEQ ID NO:Y, the polypeptide sequence as defined in column 7 of Table 1A, a polypeptide sequence encoded by the polynucleotide sequence in SEQ ID NO:X, a polypeptide sequence encoded by the nucleotide sequence as defined in columns 8 and 9 of Table 2, a polypeptide sequence encoded by the nucleotide sequence as defined in column 6 of Table 1B, a polypeptide sequence encoded by the complement of the polynucleotide sequence in SEQ ID NO:X, and/or a polypeptide sequence encoded by the cDNA sequence contained in Clone ID NO:Z.

[84] "Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

[85] Thus, one aspect of the invention provides an isolated nucleic acid molecule comprising, or alternatively consisting of, a polynucleotide having a nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence described in SEQ ID NO:X or contained in the cDNA sequence of Clone ID NO:Z; (b) a nucleotide sequence in SEQ ID NO:X or the cDNA in Clone ID NO:Z which encodes the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Clone ID NO:Z; (c) a nucleotide sequence in SEQ ID NO:X or the cDNA in Clone ID NO:Z which encodes a mature polypeptide; (d) a nucleotide sequence in SEQ ID NO:X or the cDNA sequence of Clone ID NO:Z, which encodes a biologically active fragment of a polypeptide; (e) a nucleotide sequence in SEQ ID NO:X or the cDNA sequence of Clone ID NO:Z, which encodes an antigenic fragment of a polypeptide; (f) a nucleotide sequence encoding a polypeptide comprising the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Clone ID NO:Z; (g) a nucleotide sequence encoding a mature polypeptide of the amino acid sequence of SEQ ID NO:Y or the amino acid sequence encoded by the cDNA in Clone ID NO:Z; (h) a nucleotide sequence encoding a biologically active fragment of a polypeptide having the complete amino acid sequence of SEO ID NO:Y or the complete amino acid sequence encoded by the cDNA in Clone ID NO:Z; (i) a nucleotide sequence encoding an antigenic fragment of a polypeptide having the complete amino acid sequence of SEQ ID NO:Y or the complete

amino acid sequence encoded by the cDNA in Clone ID NO:Z; and (j) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), or (i) above.

[86] The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), or (j) above, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence of the cDNA contained in Clone ID NO:Z or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEO ID NO:X, a polypeptide sequence encoded by the complement of the polynucleotide sequence in SEQ ID NO:X, a nucleotide sequence encoding the polypeptide encoded by the cDNA contained in Clone ID NO:Z, the nucleotide coding sequence in SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or the complementary strand thereto, a nucleotide sequence encoding the polypeptide encoded by the nucleotide sequence in SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or the complementary strand thereto, the nucleotide coding sequence in SEQ ID NO:B as defined in column 6 of Table 1B or the complementary strand thereto, a nucleotide sequence encoding the polypeptide encoded by the nucleotide sequence in SEQ ID NO:B as defined in column 6 of Table 1B or the complementary strand thereto, the nucleotide sequence in SEQ ID NO:X encoding the polypeptide sequence as defined in column 7 of Table 1A or the complementary strand thereto, nucleotide sequences encoding the polypeptide as defined in column 7 of Table 1A or the complementary strand thereto, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides and nucleic acids.

[87] In a preferred embodiment, the invention encompasses nucleic acid molecules which comprise, or alternatively, consist of a polynucleotide which hybridizes under stringent hybridization conditions, or alternatively, under lower stringency conditions, to a polynucleotide in (a), (b), (c), (d), (e), (f), (g), (h), or (i), above, as are polypeptides encoded by these polynucleotides. In another preferred embodiment, polynucleotides which

hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions, or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

In another embodiment, the invention provides a purified protein comprising, or alternatively consisting of, a polypeptide having an amino acid sequence selected from the group consisting of: (a) the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Clone ID NO:Z; (b) the amino acid sequence of a mature form of a polypeptide having the amino acid sequence of SEQ ID NO:Y or the amino acid sequence encoded by the cDNA in Clone ID NO:Z; (c) the amino acid sequence of a biologically active fragment of a polypeptide having the complete amino acid sequence encoded by the cDNA in Clone ID NO:Z; and (d) the amino acid sequence of an antigenic fragment of a polypeptide having the complete amino acid sequence of SEQ ID NO:Y or the complete amino acid sequence encoded by the cDNA in Clone ID NO:Y or the complete amino acid sequence encoded by the cDNA in Clone ID NO:Y or the complete

[89] The present invention is also directed to proteins which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, any of the amino acid sequences in (a), (b), (c), or (d), above, the amino acid sequence shown in SEQ ID NO:Y, the amino acid sequence encoded by the cDNA contained in Clone ID NO:Z, the amino acid sequence of the polypeptide encoded by the nucleotide sequence in SEQ ID NO:X as defined in columns 8 and 9 of Table 2, the amino acid sequence of the polypeptide encoded by the nucleotide sequence in SEQ ID NO:B as defined in column 6 of Table 1B, the amino acid sequence as defined in column 7 of Table 1A, an amino acid sequence encoded by the nucleotide sequence in SEQ ID NO:X, and an amino acid sequence encoded by the complement of the polynucleotide sequence in SEQ ID NO:X. Fragments of these polypeptides are also provided (e.g., those fragments described herein). Further proteins encoded by polynucleotides which hybridize to the complement of the nucleic acid molecules encoding these amino acid sequences under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are the polynucleotides encoding these proteins.

[90] By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the

nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be an entire sequence referred to in Table 1A or 2 as the ORF (open reading frame), or any fragment specified as described herein.

[91] As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is expressed as percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases

outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

[93] For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

[94] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[95] As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence of a polypeptide referred to in Table 1A (e.g., the amino acid sequence identified in column 6) or Table 2 (e.g., the amino acid sequence of the polypeptide encoded by the polynucleotide

sequence defined in columns 8 and 9 of Table 2) or a fragment thereof, the amino acid sequence of the polypeptide encoded by the polynucleotide sequence in SEQ ID NO:B as defined in column 6 of Table 1B or a fragment thereof, the amino acid sequence of the polypeptide encoded by the nucleotide sequence in SEQ ID NO:X or a fragment thereof, or the amino acid sequence of the polypeptide encoded by cDNA contained in Clone ID NO:Z, or a fragment thereof, can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237-245 (1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is expressed as percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

[96] If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and Cterminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C- terminal residues of the subject sequence.

[97] For example, a 90 amino acid residue subject sequence is aligned with a 100

residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

[98] The polynucleotide variants of the invention may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, polypeptide variants in which less than 50, less than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

[99] Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

[100] Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-

terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. As an example, Ron et al. (J. Biol. Chem. 268: 2984-2988 (1993)) reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

[101] Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem. 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

[102] Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N-or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

[103] Thus, the invention further includes polypeptide variants which show a functional activity (e.g., biological activity) of the polypeptides of the invention. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

[104] The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, (e.g., encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion), irrespective of whether they encode a polypeptide having functional activity. This is because even where a particular nucleic acid molecule does not encode a

polypeptide having functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having functional activity include, inter alia, (1) isolating a gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); (3) Northern Blot analysis for detecting mRNA expression in specific tissues (e.g., normal or diseased tissues); and (4) in situ hybridization (e.g., histochemistry) for detecting mRNA expression in specific tissues (e.g., normal or diseased tissues).

Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having functional activity. By a polypeptide having "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide of the invention for binding) to an anti-polypeptide of the invention antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide of the invention.

[106] The functional activity of the polypeptides, and fragments, variants and derivatives of the invention, can be assayed by various methods.

[107] For example, in one embodiment where one is assaying for the ability to bind or compete with a full-length polypeptide of the present invention for binding to an antipolypetide antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation

assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

[108] In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky et al., Microbiol. Rev. 59:94-123 (1995). In another embodiment, the ability of physiological correlates of a polypeptide of the present invention to bind to a substrate(s) of the polypeptide of the invention can be routinely assayed using techniques known in the art.

[109] In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the present invention and fragments, variants and derivatives thereof to elicit polypeptide related biological activity (either *in vitro* or *in vivo*). Other methods will be known to the skilled artisan and are within the scope of the invention.

[110] Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to, for example, the nucleic acid sequence of the cDNA contained in Clone ID NO:Z, the nucleic acid sequence referred to in Table 1A (SEQ ID NO:X), the nucleic acid sequence disclosed in Table 2 (e.g., the nucleic acid sequence delineated in columns 8 and 9) or fragments thereof, will encode polypeptides "having functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as

further described below.

[111] For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

[112] The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

[113] The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. See Cunningham and Wells, Science 244:1081-1085 (1989). The resulting mutant molecules can then be tested for biological activity.

[114] As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitutions with one or more of the

amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, serum albumin (preferably human serum albumin) or a fragment thereof, or leader or secretory sequence, or a sequence facilitating purification, or (v) fusion of the polypeptide with another compound, such as albumin (including but not limited to recombinant albumin (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

- [115] For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. See Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).
- [116] A further embodiment of the invention relates to polypeptides which comprise the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions from a polypeptide sequence disclosed herein. Of course it is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a polypeptide of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X, an amino acid sequence encoded by the portion of SEQ ID NO:X as defined in columnns 8 and 9 of Table 2, an amino acid sequence encoded by the complement of SEQ ID NO:X, and/or an amino acid sequence encoded by cDNA contained in Clone ID NO:Z which contains, in order of ever-increasing preference, at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 amino acid substitutions.
- [117] In specific embodiments, the polypeptides of the invention comprise, or alternatively, consist of, fragments or variants of a reference amino acid sequence selected from: (a) the amino acid sequence of SEQ ID NO:Y or fragments thereof (e.g., the mature

form and/or other fragments described herein); (b) the amino acid sequence encoded by SEQ ID NO:X or fragments thereof; (c) the amino acid sequence encoded by the complement of SEQ ID NO:X or fragments thereof; (d) the amino acid sequence encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or fragments thereof; and (e) the amino acid sequence encoded by cDNA contained in Clone ID NO:Z or fragments thereof; wherein the fragments or variants have 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, amino acid residue additions, substitutions, and/or deletions when compared to the reference amino acid sequence. In preferred embodiments, the amino acid substitutions are conservative. Polynucleotides encoding these polypeptides are also encompassed by the invention.

## Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the [118] polynucleotides (nucleic acids) of the invention. In the present invention, a "polynucleotide fragment" refers to a polynucleotide having a nucleic acid sequence which, for example: is a portion of the cDNA contained in Clone ID NO:Z or the complementary strand thereto; is a portion of the polynucleotide sequence encoding the polypeptide encoded by the cDNA contained in Clone ID NO:Z or the complementary strand thereto; is a portion of a polynucleotide sequence encoding the amino acid sequence encoded by the region of SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or the complementary strand thereto; is a portion of the polynucleotide sequence of SEQ ID NO:X as defined in columns 8 and 9 of Table 2 or the complementary strand thereto; is a portion of the polynucleotide sequence in SEO ID NO:X or the complementary strand thereto; is a polynucleotide sequence encoding a portion of the polypeptide of SEQ ID NO:Y; is a polynucleotide sequence encoding a portion of a polypeptide encoded by SEQ ID NO:X; is a polynucleotide sequence encoding a portion of a polypeptide encoded by the complement of the polynucleotide sequence in SEQ ID NO:X; is a portion of a polynucleotide sequence encoding the amino acid sequence encoded by the region of SEQ ID NO:B as defined in column 6 of Table 1B or the complementary strand thereto; or is a portion of the polynucleotide sequence of SEQ ID NO:B as defined in column 6 of Table 1B or the complementary strand thereto.

[119] The polynucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, or at least

about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in Clone ID NO:Z, or the nucleotide sequence shown in SEQ ID NO:X or the complementary stand thereto. In this context "about" includes the particularly recited value or a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., at least 160, 170, 180, 190, 200, 250, 500, 600, 1000, or 2000 nucleotides in length ) are also encompassed by the invention.

Moreover, representative examples of polynucleotide fragments of the invention [120] comprise, or alternatively consist of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, 3551-3600, 3601-3650, 3651-3700, 3701-3750, 3751-3800, 3801-3850, 3851-3900, 3901-3950, 3951-4000, 4001-4050, 4051-4100, 4101-4150, 4151-4200, 4201-4250, 4251-4300, 4301-4350, 4351-4400, 4401-4450, 4451-4500, 4501-4550, 4551-4600, 4601-4650, 4651-4700, 4701-4750, 4751-4800, 4801-4850, 4851-4900, 4901-4950, 4951-5000, 5001-5050, 5051-5100, 5101-5150, 5151-5200, 5201-5250, 5251-5300, 5301-5350, 5351-5400, 5401-5450, 5451-5500, 5501-5550, 5551-5600, 5601-5650, 5651-5700, 5701-5750, 5751-5800, 5801-5850, 5851-5900, 5901-5950, 5951-6000, 6001-6050, 6051-6100, 6101-6150, 6151-6200, 6201-6250, 6251-6300, 6301-6350, 6351-6400, 6401-6450, 6451-6500, 6501-6550, 6551-6600, 6601-6650, 6651-6700, 6701-6750, 6751-6800, 6801-6850, 6851-6900, 6901-6950, 6951-7000, 7001-7050, 7051-7100, 7101-7150, 7151-7200, 7201-7250, 7251-7300 or 7301 to the end of SEO ID NO:X, or the complementary strand thereto. In this context "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a

polypeptide which has a functional activity (e.g., biological activity). More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

[121] Further representative examples of polynucleotide fragments of the invention comprise, or alternatively consist of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 601-650, 651-700, 701-750, 751-800, 801-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, 3551-3600, 3601-3650, 3651-3700, 3701-3750, 3751-3800, 3801-3850, 3851-3900, 3901-3950, 3951-4000, 4001-4050, 4051-4100, 4101-4150, 4151-4200, 4201-4250, 4251-4300, 4301-4350, 4351-4400, 4401-4450, 4451-4500, 4501-4550, 4551-4600, 4601-4650, 4651-4700, 4701-4750, 4751-4800, 4801-4850, 4851-4900, 4901-4950, 4951-5000, 5001-5050, 5051-5100, 5101-5150, 5151-5200, 5201-5250, 5251-5300, 5301-5350, 5351-5400, 5401-5450, 5451-5500, 5501-5550, 5551-5600, 5601-5650, 5651-5700, 5701-5750, 5751-5800, 5801-5850, 5851-5900, 5901-5950, 5951-6000, 6001-6050, 6051-6100, 6101-6150, 6151-6200, 6201-6250, 6251-6300, 6301-6350, 6351-6400, 6401-6450, 6451-6500, 6501-6550, 6551-6600, 6601-6650, 6651-6700, 6701-6750, 6751-6800, 6801-6850, 6851-6900, 6901-6950, 6951-7000, 7001-7050, 7051-7100, 7101-7150, 7151-7200, 7201-7250, 7251-7300 or 7301 to the end of the cDNA sequence contained in Clone ID NO:Z, or the complementary strand thereto. In this context "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a functional activity (e.g., biological activity). More preferably, these polynucleotides can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these polynucleotides under

stringent hybridization conditions or alternatively, under lower stringency conditions are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

Moreover, representative examples of polynucleotide fragments of the invention [122] comprise, or alternatively consist of, a nucleic acid sequence comprising one, two, three, four, five, six, seven, eight, nine, ten, or more of the above described polynucleotide fragments of the invention in combination with a polynucleotide sequence delineated in Table 1B column 6. Additional, representative examples of polynucleotide fragments of the invention comprise, or alternatively consist of, a nucleic acid sequence comprising one, two, three, four, five, six, seven, eight, nine, ten, or more of the above described polynucleotide fragments of the invention in combination with a polynucleotide sequence that is the complementary strand of a sequence delineated in column 6 of Table 1B. further embodiments, the above-described polynucleotide fragments of the invention comprise, or alternatively consist of, sequences delineated in Table 1B, column 6, and have a nucleic acid sequence which is different from that of the BAC fragment having the sequence disclosed in SEQ ID NO:B (see Table 1B, column 5). In additional embodiments, the above-described polynucleotide fragments of the invention comprise, or alternatively consist of, sequences delineated in Table 1B, column 6, and have a nucleic acid sequence which is different from that published for the BAC clone identified as BAC ID NO:A (see Table 1B, column 4). In additional embodiments, the above-described polynucleotides of the invention comprise, or alternatively consist of, sequences delineated Table 1B, column 6, and have a nucleic acid sequence which is different from that contained in the BAC clone identified as BAC ID NO:A (see Table 1B, column 4). Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides and polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more fragments of the sequences delineated in column 6 of Table 1B, and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1B, column 2) or fragments or variants thereof. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

[124] In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more fragments of the sequences delineated in column 6 of Table 1B which correspond to the same Clone ID NO:Z (see Table 1B, column 1), and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1A or 1B) or fragments or variants thereof. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

[125] In further specific embodiments, polynucleotides of the invention comprise, or alternatively consist of, one, two, three, four, five, six, seven, eight, nine, ten, or more fragments of the sequences delineated in the same row of column 6 of Table 1B, and the polynucleotide sequence of SEQ ID NO:X (e.g., as defined in Table 1A or 1B) or fragments or variants thereof. Polypeptides encoded by these polynucleotides, other polynucleotides that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B and the 5' 10 polynucleotides of the sequence of SEQ ID NO:X are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids that encode these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In additional specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B and the 5' 10 polynucleotides of a fragment or variant of the sequence of SEQ ID NO:X (e.g., as described herein) are directly contiguous Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by

these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

In further specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of a fragment or variant of the sequence of SEQ ID NO:X and the 5' 10 polynucleotides of the sequence of one of the sequences delineated in column 6 of Table 1B are directly contiguous. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

[129] In specific embodiments, polynucleotides of the invention comprise, or alternatively consist of a polynucleotide sequence in which the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B and the 5' 10 polynucleotides of another sequence in column 6 are directly contiguous. In preferred embodiments, the 3' 10 polynucleotides of one of the sequences delineated in column 6 of Table 1B is directly contiguous with the 5' 10 polynucleotides of the next sequential exon delineated in Table 1B, column 6. Nucleic acids which hybridize to the complement of these 20 contiguous polynucleotides under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention. Polypeptides encoded by these polynucleotides and/or nucleic acids, other polynucleotides and/or nucleic acids encoding these polypeptides, and antibodies that bind these polypeptides are also encompassed by the invention. Additionally, fragments and variants of the above-described polynucleotides, nucleic acids, and polypeptides are also encompassed by the invention.

[130] In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y, a portion of an amino acid sequence encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, a portion of an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO:X, a portion of an amino acid sequence encoded by the complement of the

polynucleotide sequence in SEQ ID NO:X, and/or a portion of an amino acid sequence encoded by the cDNA contained in Clone ID NO:Z. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention; include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 101-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760, 761-780, 781-800, 801-820, 821-840, 841-860, 861-880, 881-900, 901-920, 921-940, 941-960, 961-980, 981-1000, 1001-1020, 1021-1040, 1041-1060, 1061-1080, 1081-1100, 1101-1120, 1121-1140, 1141-1160, 1161-1180, 1181-1200, 1201-1220, 1221-1240, 1241-1260, 1261-1280, 1281-1300, 1301-1320, 1321-1340, 1341-1360, 1361-1380, 1381-1400, 1401-1420, 1421-1440, or 1441 to the end of the coding region of cDNA and SEQ ID NO: Y. In a preferred embodiment, polypeptide fragments of the invention include, for example, fragments comprising, or alternatively consisting of, from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 101-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760, 761-780, 781-800, 801-820, 821-840, 841-860, 861-880, 881-900, 901-920, 921-940, 941-960, 961-980, 981-1000, 1001-1020, 1021-1040, 1041-1060, 1061-1080, 1081-1100, 1101-1120, 1121-1140, 1141-1160, 1161-1180, 1181-1200, 1201-1220, 1221-1240, 1241-1260, 1261-1280, 1281-1300, 1301-1320, 1321-1340, 1341-1360, 1361-1380, 1381-1400, 1401-1420, 1421-1440, or 1441 to the end of the coding region of SEQ ID NO:Y. Moreover, polypeptide fragments of the invention may be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, or ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

[131] Even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other

functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[132] Accordingly, polypeptide fragments include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

The present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X or the complement thereof, a polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, a polypeptide encoded by the portion of SEQ ID NO:B as defined in column 6 of Table 1B, and/or a polypeptide encoded by the cDNA contained in Clone ID NO:Z). In particular, N-terminal deletions may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y, or the polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2), and m is defined as any integer ranging from 2 to q-6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, a polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, and/or a polypeptide encoded by the cDNA contained in Clone ID NO:Z). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of amino acid residue in a polypeptide of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[135] In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a polypeptide encoded by SEQ ID NO:X (e.g., including, but not limited to, the preferred polypeptide disclosed as SEQ ID NO:Y and the polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2), the cDNA contained in Clone ID NO:Z, and/or the complement thereof, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example the ability of the shortened mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

[137] The present application is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence set

forth herein. In preferred embodiments, the application is directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to polypeptides having the amino acid sequence of the specific N- and C-terminal deletions. Polynucleotides encoding these polypeptides are also encompassed by the invention.

[138] Any polypeptide sequence encoded by, for example, the polynucleotide sequences set forth as SEQ ID NO:X or the complement thereof, (presented, for example, in Tables 1A and 2), the cDNA contained in Clone ID NO:Z, or the polynucleotide sequence as defined in column 6 of Table 1B, may be analyzed to determine certain preferred regions of the polypeptide. For example, the amino acid sequence of a polypeptide encoded by a polynucleotide sequence of SEQ ID NO:X (e.g., the polypeptide of SEQ ID NO:Y and the polypeptide encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2) or the cDNA contained in Clone ID NO:Z may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715 USA; http://www.dnastar.com/).

[139] Polypeptide regions that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions; Chou-Fasman alpha-regions, beta-regions, and turn-regions; Kyte-Doolittle hydrophilic regions and hydrophobic regions; Eisenberg alpha-and beta-amphipathic regions; Karplus-Schulz flexible regions; Emini surface-forming regions; and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides of the invention in this regard are those that encode polypeptides comprising regions that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above.

[140] Additionally, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from data by DNASTAR analysis by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

[141] Preferred polypeptide fragments of the invention are fragments comprising, or alternatively, consisting of, an amino acid sequence that displays a functional activity (e.g. biological activity) of the polypeptide sequence of which the amino acid sequence is a fragment. By a polypeptide displaying a "functional activity" is meant a polypeptide capable of one or more known functional activities associated with a full-length protein, such as, for example, biological activity, antigenicity, immunogenicity, and/or multimerization, as described herein.

- [142] Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.
- [143] In preferred embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the antigenic fragments of the polypeptide of SEQ ID NO:Y, or portions thereof. Polynucleotides encoding these polypeptides are also encompassed by the invention.
- [144] The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of: the polypeptide sequence shown in SEQ ID NO:Y; a polypeptide sequence encoded by SEQ ID NO:X or the complementary strand thereto; the polypeptide sequence encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2; the polypeptide sequence encoded by the portion of SEQ ID NO:B as defined in column 6 of Table 1B or the complement thereto; the polypeptide sequence encoded by the cDNA contained in Clone ID NO:Z; or the polypeptide sequence encoded by a polynucleotide that hybridizes to the sequence of SEQ ID NO:X, the complement of the sequence of SEQ ID NO:X, the complement of a portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, or the cDNA sequence contained in Clone ID NO:Z under stringent hybridization conditions or alternatively, under lower stringency hybridization as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X, or a fragment thereof), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to the complementary strand

under stringent hybridization conditions or alternatively, under lower stringency hybridization conditions defined *supra*.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described *infra*. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

[146] Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

[147] In the present invention, antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 40, at least 50, and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

[148] Non-limiting examples of epitopes of polypeptides that can be used to generate antibodies of the invention include a polypeptide comprising, or alternatively consisting of,

at least one, two, three, four, five, six or more of the portion(s) of SEQ ID NO:Y specified in column 7 of Table 1A. These polypeptide fragments have been determined to bear antigenic epitopes of the proteins of the invention by the analysis of the Jameson-Wolf antigenic index which is included in the DNAStar suite of computer programs. By "comprise" it is intended that a polypeptide contains at least one, two, three, four, five, six or more of the portion(s) of SEQ ID NO:Y shown in column 7 of Table 1A, but it may contain additional flanking residues on either the amino or carboxyl termini of the recited portion. Such additional flanking sequences are preferably sequences naturally found adjacent to the portion; i.e., contiguous sequence shown in SEQ ID NO:Y. The flanking sequence may, however, be sequences from a heterologous polypeptide, such as from another protein described herein or from a heterologous polypeptide not described herein. In particular embodiments, epitope portions of a polypeptide of the invention comprise one, two, three, or more of the portions of SEQ ID NO:Y shown in column 7 of Table 1A.

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. See, for instance, Sutcliffe et al., *supra*; Wilson et al., *supra*; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

[150] Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, *in vivo* immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., *supra*; Wilson et al., *supra*, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If *in vivo* immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-

hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 µg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides [151] of the present invention (e.g., those comprising an immunogenic or antigenic epitope) can be fused to heterologous polypeptide sequences. For example, polypeptides of the present invention (including fragments or variants thereof), may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof, resulting in chimeric polypeptides. By way of another non-limiting example, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused with albumin (including but not limited to recombinant human serum albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)). In a preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with the mature form of human serum albumin (i.e., amino acids 1 - 585 of human serum albumin as shown in Figures 1 and 2 of EP Patent 0 322 094) which is herein incorporated by reference in its entirety. In another preferred embodiment, polypeptides and/or antibodies of the present invention (including fragments or variants thereof) are fused with polypeptide fragments comprising, or alternatively consisting of, amino acid residues 1-z of human serum albumin, where z is an integer from 369 to 419, as described in U.S. Patent 5,766,883 herein incorporated by reference in its entirety. Polypeptides and/or antibodies of the present invention (including fragments or variants thereof) may be fused to either the N- or C-terminal end of the heterologous protein (e.g., immunoglobulin Fc polypeptide or human serum albumin polypeptide).

Polynucleotides encoding fusion proteins of the invention are also encompassed by the invention.

[152] Such fusion proteins as those described above may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG fusion proteins that have a disulfidelinked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995). Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin (HA) tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., 1991, Proc. Natl. Acad. Sci. USA 88:8972-897). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an aminoterminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

## Fusion Proteins

[153] Any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, polypeptides of the present invention which are shown to be secreted can be used as targeting molecules once fused to other proteins.

[154] Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

[155] In certain preferred embodiments, proteins of the invention are fusion proteins comprising an amino acid sequence that is an N and/or C- terminal deletion of a polypeptide of the invention. In preferred embodiments, the invention is directed to a fusion protein comprising an amino acid sequence that is at least 90%, 95%, 96%, 97%, 98% or 99% identical to a polypeptide sequence of the invention. Polynucleotides encoding these proteins are also encompassed by the invention.

[156] Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

[157] As one of skill in the art will appreciate that, as discussed above, polypeptides of the present invention, and epitope-bearing fragments thereof, can be combined with heterologous polypeptide sequences. For example, the polypeptides of the present invention may be fused with heterologous polypeptide sequences, for example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM) or portions thereof (CH1, CH2, CH3, and any combination thereof, including both entire domains and portions thereof), or albumin (including, but not limited to, native or recombinant human albumin or fragments or variants thereof (see, e.g., U.S. Patent No. 5,876,969, issued March 2, 1999, EP Patent 0 413 622, and U.S. Patent No. 5,766,883, issued June 16, 1998, herein incorporated by reference in their entirety)), resulting in chimeric polypeptides. For example, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties (EP-A

0232 262). Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

[158] Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a polypeptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)).

[159] Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts,

domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[160] Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

## Recombinant and Synthetic Production of Polypeptides of the Invention

- [161] The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by synthetic and recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.
- [162] The polynucleotides of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.
- [163] The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.
- As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418, glutamine synthase, or neomycin resistance for eukaryotic cell culture, and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells

such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

[166] Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g., Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657, which are hereby incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors can be obtained from Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington et al., Bio/technology 10:169(1992) and in Biblia and Robinson Biotechnol. Prog. 11:1 (1995) which are herein incorporated by reference.

[167] The present invention also relates to host cells containing the above-described vector constructs described herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a

prokaryotic cell, such as a bacterial cell. A host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the desired modifications and processing of the foreign protein expressed.

[168] Introduction of the nucleic acids and nucleic acid constructs of the invention into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., the coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., US Patent Number 5,641,670, issued June 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[170] Polypeptides of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography,

hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

[171] Polypeptides of the present invention can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured; products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

[172] In one embodiment, the yeast Pichia pastoris is used to express polypeptides of the invention in a eukaryotic system. Pichia pastoris is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O₂. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, Pichia pastoris must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O₂. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (AOXI) is highly active. In the presence of methanol, alcohol oxidase produced from the AOXI gene comprises up to approximately 30% of the total soluble protein in Pichia pastoris. See Ellis, S.B., et al., Mol. Cell. Biol. 5:1111-21 (1985); Koutz, P.J, et al., Yeast 5:167-77 (1989); Tschopp, J.F., et al., Nucl. Acids Res. 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the AOXI regulatory sequence is expressed at exceptionally high levels in Pichia yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

- [174] Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.
- [175] In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.
- In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

[177] In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., Nature, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the Disomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, tbutylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

[178] The invention encompasses polypeptides of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH₄; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

[179] Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

[180] Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic

group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine (¹²¹I, ¹²³I, ¹²⁵I, ¹³¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (¹¹¹In, ¹¹²In, ^{113m}In, ^{115m}In), technetium (⁹⁹Tc, ^{99m}Tc), thallium (²⁰¹Ti), gallium (⁶⁸Ga, ⁶⁷Ga), palladium (¹⁰³Pd), molybdenum (⁹⁹Mo), xenon (¹³³Xe), fluorine (¹⁸F), ¹⁵³Sm, ¹⁷⁷Lu, ¹⁵⁹Gd, ¹⁴⁹Pm, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁶⁶Ho, ⁹⁰Y, ⁴⁷Sc, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁴²Pr, ¹⁰⁵Rh, and ⁹⁷Ru.

In specific embodiments, a polypeptide of the present invention or fragment or variant thereof is attached to macrocyclic chelators that associate with radiometal ions, including but not limited to, ¹⁷⁷Lu, ⁹⁰Y, ¹⁶⁶Ho, and ¹⁵³Sm, to polypeptides. In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators is ¹¹¹In. In another preferred embodiment, the radiometal ion associated with the macrocyclic chelator is ⁹⁰Y. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N''',-tetraacetic acid (DOTA). In other specific embodiments, DOTA is attached to an antibody of the invention or fragment thereof via a linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin Cancer Res. 4(10):2483-90 (1998); Peterson et al., Bioconjug. Chem. 10(4):553-7 (1999); and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50 (1999); which are hereby incorporated by reference in their entirety.

As mentioned, the proteins of the invention may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Polypeptides of the invention may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond

formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

[183] Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

[185] As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo et al., Appl. Biochem. Biotechnol. 56:59-72 (1996); Vorobjev et al., Nucleosides Nucleotides 18:2745-2750 (1999); and Caliceti et al., Bioconjug. Chem. 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, such as, for example, the method disclosed in EP 0 401 384 (coupling PEG to G-CSF), herein incorporated by reference; see also Malik et al., Exp. Hematol. 20:1028-1035 (1992), reporting pegylation of GM-CSF using tresyl chloride. For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

[187] As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

[188] One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if

necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

[189] As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992); Francis et al., Intern. J. of Hematol. 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

[190] One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride (ClSO₂CH₂CF₃). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoreothane sulphonyl group.

[191] Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-pnitrophenolcarbonate, and various MPEG-succinate derivatives. A number of additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in International Publication No. WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products

produced using the reaction chemistries set out herein are included within the scope of the invention.

[192] The number of polyethylene glycol moieties attached to each protein of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992).

[193] The polypeptides of the invention can be recovered and purified from chemical synthesis and recombinant cell cultures by standard methods which include, but are not limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

[194] The polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

[195] Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer refers to a multimer containing only polypeptides corresponding to a protein of the invention (e.g., the amino acid sequence of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X or the complement of SEQ ID NO:X, the amino acid sequence encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, and/or an amino acid sequence encoded by cDNA contained in Clone ID NO:Z (including fragments, variants, splice variants, and fusion proteins, corresponding to

these as described herein)). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing two polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing three polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

[196] As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer, a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

[197] Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked by, for example, liposome formation. Thus, in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers, are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as, for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:Y, encoded by the portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, and/or encoded by the cDNA contained in Clone ID NO:Z). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino

acid residues contained in the heterologous polypeptide sequence in a fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for example, osteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

[199] Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides

derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

[200] In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention containing Flag® polypeptide sequence. In a further embodiment, proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

[201] The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more intermolecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the Cterminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

[202] Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-

terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hydrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

## **Antibodies**

[203] Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of the invention (e.g., a polypeptide or fragment or variant of the amino acid sequence of SEQ ID NO:Y or a polypeptide encoded by the cDNA contained in Clone ID No:Z, and/or an epitope, of the present invention) as determined by immunoassays well known in the art for assaying specific antibody-antigen binding. Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), intracellularlymade antibodies (i.e., intrabodies), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the immunoglobulin molecules of the invention are IgG1. In other preferred embodiments, the immunoglobulin molecules of the invention are IgG4.

[204] Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also

included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

[205] The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

[206] Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, or by size in contiguous amino acid residues, or listed in the Tables and Figures. Preferred epitopes of the invention include the predicted epitopes shown in column 7 of Table 1A, as well as polynucleotides that encode these epitopes. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

[207] Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also

included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10⁻² M, 10⁻² M, 5 X 10⁻³ M, 10⁻³ M, 5 X 10⁻³  4  M,  $10^{-4}$  M, 5 X  $10^{-5}$  M,  $10^{-5}$  M, 5 X  $10^{-6}$  M,  $10^{-6}$  M, 5 X  $10^{-7}$  M,  $10^{7}$  M, 5 X  $10^{-8}$  M,  $10^{-8}$  M,  $5 \times 10^{-9} \text{ M}, 10^{-9} \text{ M}, 5 \times 10^{-10} \text{ M}, 10^{-10} \text{ M}, 5 \times 10^{-11} \text{ M}, 10^{-11} \text{ M}, 5 \times 10^{-12} \text{ M}, 10^{-12} \text{ M}, 5 \times 10^{-12} \text{ M}$  $10^{-13}$  M,  $10^{-13}$  M, 5 X  $10^{-14}$  M,  $10^{-14}$  M, 5 X  $10^{-15}$  M, or  $10^{-15}$  M.

[208] The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

[209] Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined

by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described *supra*). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

[210] The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptorligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

[211] Antibodies of the present invention may be used, for example, to purify, detect, and target the polypeptides of the present invention, including both *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have utility in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A

Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); incorporated by reference herein in its entirety.

[212] As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387; the disclosures of which are incorporated herein by reference in their entireties.

[213] The antibodies of the invention include derivatives that are modified, i.e, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

[216] Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

[217] Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

[218] Another well known method for producing both polyclonal and monoclonal human B cell lines is transformation using Epstein Barr Virus (EBV). Protocols for generating EBV-transformed B cell lines are commonly known in the art, such as, for example, the protocol outlined in Chapter 7.22 of Current Protocols in Immunology,

Coligan et al., Eds., 1994, John Wiley & Sons, NY, which is hereby incorporated in its entirety by reference. The source of B cells for transformation is commonly human peripheral blood, but B cells for transformation may also be derived from other sources including, but not limited to, lymph nodes, tonsil, spleen, tumor tissue, and infected tissues. Tissues are generally made into single cell suspensions prior to EBV transformation. Additionally, steps may be taken to either physically remove or inactivate T cells (e.g., by treatment with cyclosporin A) in B cell-containing samples, because T cells from individuals seropositive for anti-EBV antibodies can suppress B cell immortalization by EBV.

[219] In general, the sample containing human B cells is innoculated with EBV, and cultured for 3-4 weeks. A typical source of EBV is the culture supernatant of the B95-8 cell line (ATCC #VR-1492). Physical signs of EBV transformation can generally be seen towards the end of the 3-4 week culture period. By phase-contrast microscopy, transformed cells may appear large, clear, hairy and tend to aggregate in tight clusters of cells. Initially, EBV lines are generally polyclonal. However, over prolonged periods of cell cultures, EBV lines may become monoclonal or polyclonal as a result of the selective outgrowth of particular B cell clones. Alternatively, polyclonal EBV transformed lines may be subcloned (e.g., by limiting dilution culture) or fused with a suitable fusion partner and plated at limiting dilution to obtain monoclonal B cell lines. Suitable fusion partners for EBV transformed cell lines include mouse myeloma cell lines (e.g., SP2/0, X63-Ag8.653), heteromyeloma cell lines (human x mouse; e.g., SPAM-8, SBC-H20, and CB-F7), and human cell lines (e.g., GM 1500, SKO-007, RPMI 8226, and KR-4). Thus, the present invention also provides a method of generating polyclonal or monoclonal human antibodies against polypeptides of the invention or fragments thereof, comprising EBV-transformation of human B cells.

[220] Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

[221] For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional

antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

[222] As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

[223] Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including *in vivo* use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different

portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDRgrafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

[224] Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[225] Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain

immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol, 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; and 6,114,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

[226] Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

[227] Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" polypeptides of the invention using

techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing antiidiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand(s)/receptor(s). For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligand(s)/receptor(s), and thereby block its biological activity. Alternatively, antibodies which bind to and enhance polypeptide multimerization and/or binding, and/or receptor/ligand multimerization, binding and/or signaling can be used to generate anti-idiotypes that function as agonists of a polypeptide of the invention and/or its ligand/receptor. Such agonistic anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens as agonists of the polypeptides of the invention or its ligand(s)/receptor(s). For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligand(s)/receptor(s), and thereby promote or enhance its biological activity.

Intrabodies of the invention can be produced using methods known in the art, such as those disclosed and reviewed in Chen et al., Hum. Gene Ther. 5:595-601 (1994); Marasco, W.A., Gene Ther. 4:11-15 (1997); Rondon and Marasco, Annu. Rev. Microbiol. 51:257-283 (1997); Proba et al., J. Mol. Biol. 275:245-253 (1998); Cohen et al., Oncogene 17:2445-2456 (1998); Ohage and Steipe, J. Mol. Biol. 291:1119-1128 (1999); Ohage et al., J. Mol. Biol. 291:1129-1134 (1999); Wirtz and Steipe, Protein Sci. 8:2245-2250 (1999); Zhu et al., J. Immunol. Methods 231:207-222 (1999); and references cited therein.

## Polynucleotides Encoding Antibodies

[229] The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined *supra*, to polynucleotides that encode an antibody, preferably, that specifically binds to a polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y,

to a polypeptide encoded by a portion of SEQ ID NO:X as defined in columns 8 and 9 of Table 2, and/or to a polypeptide encoded by the cDNA contained in Clone ID NO:Z.

[230] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[232] Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties ), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[233] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described *supra*. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[234] In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

[235] Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038-1041 (1988)).

## Methods of Producing Antibodies

[236] The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques. Methods of producing antibodies include, but are not limited to, hybridoma technology, EBV transformation, and other methods discussed herein as well as through the use recombinant DNA technology, as discussed below.

Recombinant expression of an antibody of the invention, or fragment, derivative [237] or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[238] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light

chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[239] A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

[240] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated

individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione Stransferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[241] In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be [242] utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

[243] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein

products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

[244] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

[245] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu

and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215 (1993)); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

[246] The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availabilty of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suplliers, including, for example Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described

in Bebbington et al., Bio/technology 10:169(1992) and in Biblia and Robinson Biotechnol. Prog. 11:1 (1995) which are incorporated in their entirities by reference herein.

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[249] Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or *in vivo*, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., *supra*, and PCT publication WO 93/21232; EP 439,095;

Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452 (1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides [251] of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337-11341 (1992) (said references incorporated by reference in their entireties).

[252] As discussed, *supra*, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the *in vivo* half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. See EP 394,827; and Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. See, for example, Fountoulakis et al., J. Biochem. 270:3958-3964 (1995). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved

pharmacokinetic properties. See, for example, EP A 232,262. Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995)).

[253] Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

[254] The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or

phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99Tc.

[255] Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, a-interferon, β-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al., Int. Immunol., 6*:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an antiangiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

[257] Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

[258] Techniques for conjugating such therapeutic moiety to antibodies are well known. See, for example, Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

[259] Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

[260] An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

### *Immunophenotyping*

[261] The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. Translation products of the gene of the present invention may be useful as cell-specific markers, or more specifically as cellular markers that are differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic

beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison et al., Cell, 96:737-49 (1999)).

[262] These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

### Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., preclearing the cell lysate with sepharose beads). For further discussion regarding

immunoprecipitation protocols see, e.g., Ausubel et al., eds., (1994), Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, section 10.16.1.

Western blot analysis generally comprises preparing protein samples, [265] electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, (1994), Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, section 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, (1994), Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, section 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., 3H or 125I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., 3H or 125I) in the presence of increasing amounts of an unlabeled second antibody.

[268] Antibodies of the invention may be characterized using immunocytochemisty methods on cells (e.g., mammalian cells, such as CHO cells) transfected with a vector enabling the expression of an antigen or with vector alone using techniques commonly known in the art. Antibodies that bind antigen transfected cells, but not vector-only transfected cells, are antigen specific.

# Therapeutic Uses

[269] The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[270] In a specific and preferred embodiment, the present invention is directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more diseases, disorders, or conditions, including but not limited to: neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions., and/or as described elsewhere herein. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (e.g., antibodies directed to the full length protein expressed on the cell surface of a mammalian cell; antibodies directed to an epitope of a polypeptide of the invention (such as, for example, a predicted linear epitope shown in column 7 of Table 1A; or a conformational epitope, including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

[271] A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

[272] The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

[273] The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5 X 10⁻² M, 10⁻² M, 5 X 10⁻³ M, 10⁻³ M, 5 X 10⁻⁴ M, 10⁻⁴ M, 5 X 10⁻⁵ M, 10⁻⁵ M, 5 X 10⁻⁶ M, 10⁻⁶ M, 5 X 10⁻¹⁰ M, 5 X 10⁻¹⁰ M, 5 X 10⁻¹¹ M, 5 X 10⁻¹² M, 10⁻¹² M, 5 X 10⁻¹³ M, 10⁻¹³ M, 5 X 10⁻¹⁴ M, 10⁻¹⁴ M, 5 X 10⁻¹⁵ M, and 10⁻¹⁵ M.

## Gene Therapy

[275] In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

[276] Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

[277] For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH

11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990).

[278] In a preferred embodiment, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

[279] Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then transplanted into the patient. These two approaches are known, respectively, as *in vivo* or ex vivo gene therapy.

[280] In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be

used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted *in vivo* for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143- 155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234

(1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

[283] Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

[284] Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration *in vivo* of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

[286] The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

[287] Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as T lymphocytes, B lymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic

stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

[288] In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

[289] In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

[290] In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by the presence or absence of an appropriate inducer of transcription.

#### Demonstration of Therapeutic or Prophylactic Activity

[291] The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

[292] The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably a polypeptide or antibody of the invention. In a preferred embodiment, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

[293] Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

[294] Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[295] In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes,

such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

[296] In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

[297] In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

[298] Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

[300] The present invention also provides pharmaceutical compositions. compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate. Examples of suitable pharmaceutical carriers are described in "Remington's etc. Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

[301] In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a

hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[302] The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

[303] The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

[304] For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

[305] The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of

pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

# Diagnosis and Imaging

[306] Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[308] Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine

(125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[309] One facet of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[311] Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

[312] In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

[313] Presence of the labeled molecule can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

[314] In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

[315] The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

[316] In another specific embodiment of the present invention, the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that

does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

[317] In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

[318] In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

[319] In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

[320] The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific

adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

[321] Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound antiantigen antibody.

### Uses of the Polynucleotides

[322] Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

[323] The polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome, thus each polynucleotide of the present invention can routinely be used as a chromosome marker using techniques known in the art. Table 1A, column 9 provides the chromosome location of some of the polynucleotides of the invention.

[324] Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably at least 15 bp (e.g., 15-25 bp) from the sequences shown in SEQ ID NO:X. Primers can optionally be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to SEQ ID NO:X will yield an amplified fragment.

[325] Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies

that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

[326] Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

[327] For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

[328] Thus, the present invention also provides a method for chromosomal localization which involves (a) preparing PCR primers from the polynucleotide sequences in Table 1A and/or Table 2 and SEQ ID NO:X and (b) screening somatic cell hybrids containing individual chromosomes.

The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g. Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

[330] Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library)). Column 10 of Table 1A provides an OMIM reference identification number of diseases associated with the cytologic band disclosed in column 9 of Table 1A, as determined using techniques described herein and by reference to Table 5. Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a

chromosomal region associated with the disease could be one of 50-500 potential causative genes.

[331] Thus, once coinheritance is established, differences in a polynucleotide of the invention and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

[332] Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using the polynucleotides of the invention. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker. Diagnostic and prognostic methods, kits and reagents encompassed by the present invention are briefly described below and more thoroughly elsewhere herein (see e.g., the sections labeled "Antibodies", "Diagnostic Assays", and "Methods for Detecting Diseases").

[333] Thus, the invention also provides a diagnostic method useful during diagnosis of a disorder, involving measuring the expression level of polynucleotides of the present invention in cells or body fluid from an individual and comparing the measured gene expression level with a standard level of polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a disorder. Additional non-limiting examples of diagnostic methods encompassed by the present invention are more thoroughly described elsewhere herein (see, e.g., Example 12).

[334] In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the invention, where each probe has one strand containing a 31'mer-end internal to the region. In a further

embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

[335] Where a diagnosis of a related disorder, including, for example, diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed polynucleotide of the invention expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of polynucleotides of the invention" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the invention or the level of the mRNA encoding the polypeptide of the invention in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the related disorder or being determined by averaging levels from a population of individuals not having a related disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[337] By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains polypeptide of the present invention or the corresponding mRNA. As indicated, biological samples include body fluids (such as semen, lymph, vaginal pool, sera, plasma, urine, synovial fluid and spinal fluid) which contain the polypeptide of the present invention, and tissue sources found to express the polypeptide of the present invention. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[338] The method(s) provided above may preferably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides of the invention are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with polynucleotides of the invention attached may be used to identify polymorphisms between the isolated polynucleotide sequences of the invention, with polynucleotides

isolated from a test subject. The knowledge of such polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, digestive disorders, metabolic disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced *supra* are hereby incorporated by reference in their entirety herein.

[339] The present invention encompasses polynucleotides of the present invention that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides of the invention are incorporated onto a solid support, or gene chip, For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by Nielsen et al., Science 254, 1497 (1991); and Egholm et al., Nature 365, 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T.sub.m) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can be done at low ionic strengths and reduce possible interference by salt during the analysis.

[340] The compounds of the present invention have uses which include, but are not limited to, detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute

erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Gelmann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelmann et al., *supra*) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelmann et al., *supra*) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelmann et al., *supra*)

[342] For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580). However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness is not be limited to treatment, prevention, and/or prognosis of proliferative disorders of cells and tissues of hematopoietic origin, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

[343] In addition to the foregoing, a polynucleotide of the present invention can be used to control gene expression through triple helix formation or through antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press,

Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press. Boca Raton, FL (1988)). Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. The oligonucleotide described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of polypeptide of the present invention antigens. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease, and in particular, for the treatment of proliferative diseases and/or conditions. Non-limiting antisense and triple helix methods encompassed by the present invention are more thoroughly described elsewhere herein (see, e.g., the section labeled "Antisense and Ribozyme (Antagonists)").

[344] Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell. Additional non-limiting examples of gene therapy methods encompassed by the present invention are more thoroughly described elsewhere herein (see, e.g., the sections labeled "Gene Therapy Methods", and Examples 16, 17 and 18).

[345] The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This

method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

[346] The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

[347] Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman and Co. (1992)). Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

[348] There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers prepared from the sequences of the present invention, specific to tissues, including but not limited to those shown in Table 1A. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination. Additional non-limiting examples of such uses are further described herein.

[349] The polynucleotides of the present invention are also useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the

tissue(s) (e.g., immunohistochemistry assays) or cell type(s) (e.g., immunocytochemistry assays). In addition, for a number of disorders of the above tissues or cells, significantly higher or lower levels of gene expression of the polynucleotides/polypeptides of the present invention may be detected in certain tissues (e.g., tissues expressing polypeptides and/or polynucleotides of the present invention, for example, those disclosed in column 8 of Table 1A, and/or cancerous and/or wounded tissues) or bodily fluids (e.g., semen, lymph, vaginal pool, serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

[350] Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying gene expression level in cells or body fluid of an individual; (b) comparing the gene expression level with a standard gene expression level, whereby an increase or decrease in the assayed gene expression level compared to the standard expression level is indicative of a disorder.

[351] In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

### Uses of the Polypeptides

[352] Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

[353] Polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

[354] Antibodies can be used to assay levels of polypeptides encoded by polynucleotides of the invention in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based

methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (¹³¹I, ¹²⁵I, ¹²³I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (^{115m}In, ^{113m}In, ¹¹²In, ¹¹¹In), and technetium (⁹⁹Tc, ^{99m}Tc), thallium (²⁰¹Ti), gallium (⁶⁸Ga, ⁶⁷Ga), palladium (¹⁰³Pd), molybdenum (⁹⁹Mo), xenon (¹³³Xe), fluorine (¹⁸F), ¹⁵³Sm, ¹⁷⁷Lu, ¹⁵⁹Gd, ¹⁴⁹Pm, ¹⁴⁰La, ¹⁷⁵Yb, ¹⁶⁶Ho, ⁹⁰Y, ⁴⁷Sc, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁴²Pr, ¹⁰⁵Rh, ⁹⁷Ru; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[355] In addition to assaying levels of polypeptide of the present invention in a biological sample, proteins can also be detected *in vivo* by imaging. Antibody labels or markers for *in vivo* imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

[356] A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ¹³¹I, ¹¹²In, ^{99m}Tc, (¹³¹I, ¹²⁵I, ¹²³I, ¹²¹I), carbon (¹⁴C), sulfur (³⁵S), tritium (³H), indium (^{115m}In, ^{113m}In, ¹¹²In, ¹¹¹In), and technetium (⁹⁹Tc, ^{99m}Tc), thallium (²⁰¹Ti), gallium (⁶⁸Ga, ⁶⁷Ga), palladium (103Pd), molybdenum (99Mo), xenon (133Xe), fluorine (18F, 153Sm, 177Lu, 159Gd, 149Pm, 140La, ¹⁷⁵Yb, ¹⁶⁶Ho, ⁹⁰Y, ⁴⁷Sc, ¹⁸⁶Re, ¹⁸⁸Re, ¹⁴²Pr, ¹⁰⁵Rh, ⁹⁷Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which express the polypeptide encoded by a polynucleotide of the invention. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in Tumor Imaging: The Radiochemical

Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., polypeptides encoded by polynucleotides of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

[358] In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention in association with toxins or cytotoxic prodrugs.

[359] By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, Pseudomonas exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ²¹³Bi, or other radioisotopes such as, for example, ¹⁰³Pd, ¹³³Xe, ¹³¹I, ⁶⁸Ge, ⁵⁷Co, ⁶⁵Zn, ⁸⁵Sr, ³²P, ³⁵S, ⁹⁰Y, ¹⁵³Sm, ¹⁵³Gd, ¹⁶⁹Yb, ⁵¹Cr, ⁵⁴Mn, ⁷⁵Se, ¹¹³Sn, ⁹⁰Yttrium, ¹¹⁷Tin, ¹⁸⁶Rhenium, ¹⁶⁶Holmium, and ¹⁸⁸Rhenium; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin. In a specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope ⁹⁰Y. In another specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the

radioisotope ¹¹¹In. In a further specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope ¹³¹I.

[360] Techniques known in the art may be applied to label polypeptides of the invention (including antibodies). Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a polypeptide of the present invention in cells or body fluid of an individual; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

[362] Moreover, polypeptides of the present invention can be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor supressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to

bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

[363] Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease (as described *supra*, and elsewhere herein). For example, administration of an antibody directed to a polypeptide of the present invention can bind, and/or neutralize the polypeptide, and/or reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

[364] At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the biological activities described herein.

### Diagnostic Assays

[365] The compounds of the present invention are useful for diagnosis, treatment, prevention and/or prognosis of various disorders in mammals, preferably humans. Such disorders include, but are not limited to, those described herein under the section heading "Biological Activities".

[366] For a number of disorders, substantially altered (increased or decreased) levels of gene expression can be detected in tissues, cells or bodily fluids (e.g., sera, plasma, urine, semen, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" gene expression level, that is, the expression level in tissues or bodily fluids from an individual not having the disorder. Thus, the invention provides a diagnostic method useful during diagnosis of a disorder, which involves measuring the expression level of the gene encoding the polypeptide in tissues, cells or body fluid from an individual and comparing the measured gene expression level with a standard gene expression level, whereby an increase or decrease in the gene expression level(s) compared to the standard is indicative of a disorder. These diagnostic assays may be performed *in vivo* or *in vitro*, such as, for example, on blood samples, biopsy tissue or autopsy tissue.

[367] The present invention is also useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed gene expression will experience a worse clinical outcome

relative to patients expressing the gene at a level nearer the standard level.

[368] In certain embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to diagnose and/or prognose diseases and/or disorders associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1A, column 8 (Tissue Distribution Library Code).

[369] By "assaying the expression level of the gene encoding the polypeptide" is intended qualitatively or quantitatively measuring or estimating the level of the polypeptide of the invention or the level of the mRNA encoding the polypeptide of the invention in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide expression level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having the disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

[370] By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source containing polypeptides of the invention (including portions thereof) or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) and tissue sources found to express the full length or fragments thereof of a polypeptide or mRNA. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

[371] Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, Anal. Biochem. 162:156-159 (1987). Levels of mRNA encoding the polypeptides of the invention are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

[372] The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of polypeptides of the invention, in a biological sample (e.g., cells and tissues), including determination of normal and abnormal levels of polypeptides. Thus, for instance, a diagnostic assay in accordance with the invention for detecting over-expression of polypeptides of the invention compared to normal control tissue samples may be used to detect the presence of tumors. Assay techniques that can be used to determine levels of a polypeptide, such as a polypeptide of the present invention in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Assaying polypeptide levels in a biological sample can occur using any art-known method.

[373] Assaying polypeptide levels in a biological sample can occur using antibody-based techniques. For example, polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (125 I, 121 I), carbon (14 C), sulfur (35 S), tritium (3H), indium (112 In), and technetium (99 m Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

[374] The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the gene of inteest (such as, for example, cancer). The protein isolation methods employed herein may, for example, be such as those described in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the gene.

[375] For example, antibodies, or fragments of antibodies, such as those described herein, may be used to quantitatively or qualitatively detect the presence of gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example,

by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[376] In a preferred embodiment, antibodies, or fragments of antibodies directed to any one or all of the predicted epitope domains of the polypeptides of the invention (shown in column 7 of Table 1A) may be used to quantitatively or qualitatively detect the presence of gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[377] In an additional preferred embodiment, antibodies, or fragments of antibodies directed to a conformational epitope of a polypeptide of the invention may be used to quantitatively or qualitatively detect the presence of gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

[378] The antibodies (or fragments thereof), and/or polypeptides of the present invention may, additionally, be employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of gene products or conserved variants or peptide fragments thereof. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or polypeptide of the present invention. The antibody (or fragment thereof) or polypeptide is preferably applied by overlaying the labeled antibody (or fragment) onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the gene product, or conserved variants or peptide fragments, or polypeptide binding, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological methods (such as staining procedures) can be modified in order to achieve such in situ detection.

[379] Immunoassays and non-immunoassays for gene products or conserved variants or peptide fragments thereof will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of binding gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

[380] The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled antibody or detectable polypeptide of the invention. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding an antigen or an antibody. Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to an antigen or antibody. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

[382] The binding activity of a given lot of antibody or antigen polypeptide may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

[383] In addition to assaying polypeptide levels or polynucleotide levels in a biological sample obtained from an individual, polypeptide or polynucleotide can also be detected *in vivo* by imaging. For example, in one embodiment of the invention, polypeptides and/or antibodies of the invention are used to image diseased cells, such as neoplasms. In another embodiment, polynucleotides of the invention (e.g., polynucleotides complementary to all or a portion of an mRNA) and/or antibodies (e.g., antibodies directed to any one or a combination of the epitopes of a polypeptide of the invention, antibodies directed to a conformational epitope of a polypeptide of the invention, or antibodies directed to the full length polypeptide expressed on the cell surface of a mammalian cell) are used to image

diseased or neoplastic cells.

[384] Antibody labels or markers for in vivo imaging of polypeptides of the invention include those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma. Where in vivo imaging is used to detect enhanced levels of polypeptides for diagnosis in humans, it may be preferable to use human antibodies or "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using techniques described herein or otherwise known in the art. For example methods for producing chimeric antibodies are known in the art. See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).

[385] Additionally, any polypeptides of the invention whose presence can be detected, can be administered. For example, polypeptides of the invention labeled with a radio-opaque or other appropriate compound can be administered and visualized *in vivo*, as discussed, above for labeled antibodies. Further, such polypeptides can be utilized for *in vitro* diagnostic procedures.

A polypeptide-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ¹³¹I, ¹¹²In, ^{99m}Tc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for a disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the antigenic protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical* 

Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

[387] With respect to antibodies, one of the ways in which an antibody of the present invention can be detectably labeled is by linking the same to a reporter enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., J. Clin. Pathol. 31:507-520 (1978); Butler, J.E., Meth. Enzymol. 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL.; Ishikawa, E. et al., (eds.), 1981, Enzyme Immunoassay, Kgaku Shoin, Tokyo). The reporter enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Reporter enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alphaglycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the reporter enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[388] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibodies or antibody fragments, it is possible to detect polypeptides through the use of a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

[389] It is also possible to label the antibody with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent

labeling compounds are fluorescein isothiocyanate, rhodamine, phycocyanin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

[390] The antibody can also be detectably labeled using fluorescence emitting metals such as ¹⁵²Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[391] The antibody also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[392] Likewise, a bioluminescent compound may be used to label the antibody of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin:

## Methods for Detecting Diseases

[393] In general, a disease may be detected in a patient based on the presence of one or more proteins of the invention and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, urine, and/or tumor biopsies) obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a disease or disorder, including cancer and/or as described elsewhere herein. In addition, such proteins may be useful for the detection of other diseases and cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level of mRNA encoding polypeptides of the invention, which is also indicative of the presence or absence of a disease or disorder, including cancer. In general, polypeptides of the invention should be present at a level that is at least three fold higher in diseased tissue than in normal tissue.

[394] There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. See, e.g., Harlow and

Lane, *supra*. In general, the presence or absence of a disease in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

[395] In a preferred embodiment, the assay involves the use of a binding agent(s) immobilized on a solid support to bind to and remove the polypeptide of the invention from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include polypeptides of the invention and portions thereof, or antibodies, to which the binding agent binds, as described above.

[396] The solid support may be any material known to those of skill in the art to which polypeptides of the invention may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S. Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for the suitable amount of time. The contact time varies with temperature, but is typically between about 1

hour and about 1 day. In general, contacting a well of plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 ug, and preferably about 100 ng to about 1 ug, is sufficient to immobilize an adequate amount of binding agent.

[397] Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (see, e.g., Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

## Gene Therapy Methods

[398] Also encompassed by the invention are gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the present invention operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

[399] Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide of the present invention ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide of the present invention. Such methods are well-known in the art. For example, see Belldegrun, A., et al., J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer Research 53: 1107-1112 (1993); Ferrantini, M. et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura, H., et al., Cancer Research 50: 5102-5106 (1990); Santodonato, L., et al., Human Gene Therapy 7:1-10 (1996); Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be

reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

[400] As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

[401] In one embodiment, the polynucleotide of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotide of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

[402] The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

[403] Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotide of the present invention.

[404] Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide

synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

[405] The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

[406] For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

[407] The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

[408] The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous

injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

[409] The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

[411] Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

[412] Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

[413] Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol

(DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

[414] For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

[415] The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca²⁺-EDTA chelation (Papahadiopoulos et al., Biochim, Biophys. Acta (1975) 394:483; Wilson et al., Cell 17:77 (1979)); ether injection (Deamer, D. and Bangham, A., Biochim. Biophys. Acta 443:629 (1976); Ostro et al., Biochem. Biophys.

Res. Commun. 76:836 (1977); Fraley et al., Proc. Natl. Acad. Sci. USA 76:3348 (1979)); detergent dialysis (Enoch, H. and Strittmatter, P., Proc. Natl. Acad. Sci. USA 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem. 255:10431 (1980); Szoka, F. and Papahadjopoulos, D., Proc. Natl. Acad. Sci. USA 75:145 (1978); Schaefer-Ridder et al., Science 215:166 (1982)), which are herein incorporated by reference.

- [416] Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.
- [417] U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 provide methods for delivering DNA-cationic lipid complexes to mammals.
- [418] In certain embodiments, cells are engineered, ex vivo or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding a polypeptide of the present invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.
- [419] The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO₄ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

[420] The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding a polypeptide of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or *in vivo*. The transduced eukaryotic cells will express a polypeptide of the present invention.

- [421] In certain other embodiments, cells are engineered, ex vivo or *in vivo*, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses a polypeptide of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al. Am. Rev. Respir. Dis.109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).
- [422] Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.
- [423] Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, ex vivo or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

- [425] For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either ex vivo or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express a polypeptide of the invention.
- [426] Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), which are herein encorporated by reference. This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.
- [427] Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable

promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

- [428] The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.
- [429] The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.
- [430] The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.
- [431] The polynucleotide encoding a polypeptide of the present invention may contain a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.
- [432] Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle

accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

- [433] A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.
- [434] Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.
- [435] Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site. In specific embodiments, suitable delivery vehicles for use with systemic administration comprise liposomes comprising polypeptides of the invention for targeting the vehicle to a particular site.
- [436] Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

[437] Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

[438] Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

## **Biological Activities**

[439] Polynucleotides or polypeptides, or agonists or antagonists of the present invention, can be used in assays to test for one or more biological activities. If these polynucleotides or polypeptides, or agonists or antagonists of the present invention, do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides, and agonists or antagonists could be used to diagnose, prognose, prevent and/or treat the associated disease.

[440] Human proteins are believed to be involved in biological activities associated with a variety of biological processes, for example, cellular signaling. Accordingly, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders associated with aberrant activity of human polypeptides.

[441] In preferred embodiments, compositions of the invention (including polynucleotides, polypeptides and antibodies of the invention, and fragments and variants thereof) may be used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders relating to diseases and disorders of the endocrine system, the nervous system (See, for example, "Neurological Disorders" section below), and the immune system (See, for example, "Immune Activity" section below).

[442] In certain embodiments, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to

diagnose and/or prognose diseases and/or disorders associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1a, column 8 (Tissue Distribution Library Code).

[443] Thus, polynucleotides, translation products and antibodies of the invention are useful in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders associated with activities that include, but are not limited to, prohormone activation, neurotransmitter activity, cellular signaling, cellular proliferation, cellular differentiation, and cell migration.

[444] More generally, polynucleotides, translation products and antibodies corresponding to this gene may be useful for the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders associated with the following systems.

## **Immune Activity**

[445] Polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention may be useful in treating, preventing, diagnosing and/or prognosing diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides, polypeptides, antibodies, and/or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

[446] In another embodiment, a polypeptide of the invention, or polynucleotides, antibodies, agonists, or antagonists corresponding to that polypeptide, may be used to treat diseases and disorders of the immune system and/or to inhibit or enhance an immune response generated by cells associated with the tissue(s) in which the polypeptide of the invention is expressed, including one, two, three, four, five, or more tissues disclosed in Table 1A, column 8 (Tissue Distribution Library Code).